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TWO IMPORTANT NEW TYPES OF CITROUS HYBRIDS FOR THE HOME GARDEN—CITRANGEQUATS AND LIMEQUATS[1]

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The breeding of cold-resistant citrous fruits suitable for culture in the southern part of the cotton belt has been in progress for many years. The citrange, a cross of the hardy Chinese trifoliate orange, *Poncirus trifoliata* (L.) Raf. and the common sweet orange, *Citrus sinensis*, Osb. was the first of the hardy hybrids secured. The citrange was first reported on by the senior author and Dr. H. J. Webber[2] in the Yearbook of the Department of Agriculture for 1904, where the Rusk and Willits citranges, two that appear below as parents of new hybrids, were described and illustrated. It soon became evident that although the citrange is much like the trifoliate orange in its resistance to extreme cold when it is in a dormant condition, it is easily forced into a new growth n spring and sometimes is severely injured by untimely cold snaps. In consequence of this imperfect resistance of the citrange to spring and early autumn frosts, about 15 years ago the effort was first made to create new types of hybrids which would partake in some measure of another type of hardiness possessed very strikingly by the kumquat oranges. The kumquat is one of the hardiest of the evergreen citrous fruit trees; besides having a fair degree of resistance to cold it has an extremely high degree of winter dormancy. Both of the species of kumquat commonly grown in this country, the round and the oval kumquats, are able to pass unaffected through spells of hot weather which force other citrous into a tender and succulent growth that is liable to be injured by a slight frost.

The first successful hybrids wherein the kumquat was used with the idea of securing increased dormancy were made by the senior author in the spring of 1909. Four principal types of these kumquat hybrids were made: First, those between the trifoliate orange and the kumquat. These proved extraordinarily hard to make, and most of them were very weak, many of them dying while still small seedlings. None of these have proved as yet to be of any considerable value. Second, hybrids were made in considerable number between the more promising citranges, especially the Rusk and Willits, already widely cultivated in the cotton belt, and the kumquats. The hybrids resulting from these crosses are known as citrangequats, and are described in the present paper. Third, the effort was made to hybridize the kumquat with the West India lime,

[1] Accepted for publication Oct. 28, 1921.

[2] WEBBER, Herbert J., and SWINGLE, Walter T. NEW CITRUS CREATIONS OF THE DEPARTMENT OF AGRICULTURE. *In* U. S. Dept. Agr. Yearbook, 1904, p. 227-235, fig. 23, pl. 11-14, 16. 1905.

the lime being the tenderest citrous fruit grown in the United States, often being injured by cold even in central or south Florida. The hybrids resulting from this cross have been called limequats, and are also described here. Fourth, the attempt was made to hybridize the kumquat with the lemon, the lemon being almost as tender as the West India lime. None of these hybrids have been sufficiently well studied to warrant reporting on them as yet. So far as observed they have fruits very similar to the limequats described below except that the peel is much thicker.

THOMASVILLE CITRANGEQUAT

Among the citrous crosses made at Eustis, Fla., in June, 1909, by the senior author, was one in which pollen of the Willits citrange was used to fertilize flowers of the oval kumquat (*Fortunella margarita*) (Lour.) Swing.

Budded plants of the best of the hybrids resulting from this cross were sent to a number of cooperators in the southeastern States as soon as it was possible to effect propagation. The first plant to bear fruit was numbered C.P.B. 48010.[3] Fruits were received at Washington, D. C., in November, 1915, produced by a plant of this number that had been sent to Mr. P. J. Hjort at Thomasville, Ga., in March, 1913. As this hybrid was first fruited at Thomasville, where it was proved sufficiently desirable as a fruit for home use to induce Mr. Hjort to propagate it in his nursery, it is proposed to call it the Thomasville citrangequat.

The Willits citrange, the pollen parent, is itself a hybrid resulting from crossing the sweet orange (*Citrus sinensis*, Osb.) with the trifoliate orange (*Poncirus trifoliata* (L.) Raf.), the former supplying the pollen which was used on the pistils of the trifoliate orange.

While this citrange is of fairly good quality and makes an excellent ade, the oil of the peel is very bitter and ill-smelling, and a single drop is enough to ruin a pitcher of ade. It was hoped by hybridizing the Willits citrange with the oval kumquat to overcome this drawback of the citrange since the peel of the kumquat is so mild as to be edible, and at the same time the juice of the kumquat is pleasantly acid, so that a hybrid between it and the citrange would be expected to be suitable for making ade. These expectations were fully justified, as is shown below.

As was noted in the introductory paragraph of this paper, another quality that it was hoped to secure in this hybrid was an increased winter dormancy over that shown by the citrange. The kumquat possesses winter dormancy of a very high degree and is able to go through long spells of warm weather without starting new growth. The new growth of practically all citrous fruits is, of course, easily injured by even moderate frosts. Although the Willits, like other hybrids of the trifoliate orange, is extremely hardy when in a dormant condition, it is not able to stand long periods of warm weather in winter without starting new growth. The new citrange-kumquat hybrid, as was hoped, is superior to the Willits in all-around hardiness and shows a distinctly higher winter dormancy. Being the result of crossing a citrange with a kumquat, the new hybrid was called a citrangequat.

The citrangequat seedlings showed from the start, phenomenal vigor and in fact made the most rapid and continuous growth of all the nu-

[3] The abbreviation C. P. B. is used for the name of the Office of Crop Physiology and Breeding Investigations of the Bureau of Plant Industry, United States Department of Agriculture. All the numbers cited in this paper are of this series.

merous rapid-growing hybrids under propagation in the department greenhouses during the winter of 1909-10.

Plate 1 shows the vigorous growth manifested by a few of these citrangequats less than one year from the time of planting the seeds, the plants being, from left to right, 57½, 61, 49, and 52 inches high, while an oval kumquat of the same age (on the extreme right) was only 24½ inches high, only half the height of the smallest of the four citrangequats.

The Thomasville citrangequat is a hybrid of the oval Kumquat, *Fortunella margarita* (Lour.) Swing, and the Willits citrange, the last named being the pollen parent. The Thomasville and other citrangequats are what is known as trigeneric hybrids—that is, they were secured by hybridizing plants belonging to three distinct genera, Citrus, Poncirus, and Fortunella, as has already been noted. In this case the citrangequat is in effect a second-generation citrange—that is, a cross of the ordinary orange and the trifoliate orange, and a first generation cross of the oval kumquat. It is, therefore, one-half kumquat and approximately one-fourth each of common orange and trifoliate orange.

It is an interesting fact that, although the kumquat is a dwarf plant, nevertheless hybrids between it and the citrange have shown distinctly more vigor than similar hybrids between citranges and grapefruit, despite the fact that the grapefruit is a very much larger plant than the kumquat.[4] The vigor of the Thomasville and some of the other citrangequats is so great that their growth often exceeds the combined growth of the three parent species.

GENERAL FRUIT AND TREE CHARACTERISTICS OF THE THOMASVILLE CITRANGEQUAT

It was evident from an examination of the first fruits borne that a new type of citrous fruit had been created, having much of the pomological character of the lime, despite the absence of any lime blood. The fruits average much larger than the kumquat and are usually as large as the lime, which they resemble more closely than any other citrous fruit. Plate 2, A, B will give a good idea of the shape and size of the fruit and the manner of its attachment to the twigs. The pronounced "gooseneck" fruit spurs and clawlike calyx points are unique characters hich make the fruit easy to identify.

The fruits are of attractive appearance, being a glossy dark green hen immature, changing to a light yellow, and finally to an orange ellow when fully mature. They develop an abundant juice content hen only half grown, a point of importance in their practical utilization. hough rather late in blooming, fruits develop very rapidly, in this respect resembling those of another hybrid, the Eustis limequat, also described in this paper. Although usually not mature until October or November, the fruits can be used for ade from July on through the summer and early autumn, when acid fruits are especially appreciated. When fully mature, they lose much of their acidity, and are sufficiently sweet to eat out of hand. By reason of its hardiness and pleasantly acid juice this new fruit makes available for home fruit gardens an ade fruit in regions much too cold for growing either lemons or limes. It has stood temperatures as low as 12° F. without injury and probably will

[4] This curious fact is perhaps explained by the fact that the kumquat is botanically very remote from the two parent species that enter into the citrange, whereas the grapefruit is closely related to one of the parents of the citrange—the common orange.

survive even lower temperatures. The evergreen nature of the tree also recommends it for home gardens or lawn planting. Judicious pruning or pinching back of long shoots will assist in giving the tree a fairly compact habit in place of its natural upright form.

The Thomasville citrangequat makes an excellent marmalade without the admixture of any other citrous fruits. The mildly pungent peel and the pleasantly flavored acid juice combine to give the marmalade an unusually good flavor, rivaling the best Dundee marmalade.

RESISTANCE TO DISEASE

The advent of Citrus-canker in the Gulf Coast States made it necessary to test any new Citrus varieties and hybrids with reference to their susceptibility to that disease before they could be recommended for culture.

As is well known, the kumquat is the most canker-resistant of all citrous fruits, its resistance amounting to practical immunity under field conditions. In this new hybrid, the Thomasville citrangequat, the kumquat parent has seemingly transmitted its remarkable canker resistance unimpaired, notwithstanding the fact that one parent of the citrangequat, the citrange, is decidedly susceptible to canker.

Several plants of the Thomasville citrangequat have been inoculated and continuously exposed to Citrus-canker for a period of four years without any canker having been developed except from needle punctures, from which canker failed to spread.[5]

It is highly important that only canker-resistant citrous fruits be propagated in the Gulf Coast States west of Florida, as a measure of insurance against a serious reinfection. From this viewpoint, the citrangequat becomes of great importance to this region, to meet the need for home-grown acid citrous fruits.

While somewhat affected by Citrus scab, the quality of the fruit is not greatly impaired thereby, and if planted away from scab-susceptible varieties it is probable that scab would not be at all serious on the citrangequat.

POSSIBLE USE OF CITRANGEQUATS AS A STOCK FOR SATSUMA ORANGES

The extraordinary vigor of growth, great hardiness, and extreme canker resistance shown by this hybrid suggested at once its possible value as a stock, especially for use in the Gulf Coast States west of Florida. In this region almost the only stock used for Satsuma and other Citrus varieties is the trifoliate orange, a very hardy, deciduous tree, closely related to the true species of Citrus. It is extremely thorny and is used extensively as a hedge plant, the fruits from these hedges providing an abundance of seeds to use in growing nursery stock. Unfortunately, the trifoliate orange is very susceptible to Citrus-canker. One necessary step in the eradication of canker is the complete elimination of these trifoliate orange hedges. The presence of large numbers of trifoliate seedlings in Citrus nurseries is also a menace in the event of a reappearance of Citrus-canker.

When considering the possible use of the citrangequat as a stock, the small number of seeds produced seemed an almost unsurmountable

[5] PELTIER, George L. SUSCEPTIBILITY AND RESISTANCE TO CITRUS-CANKER OF THE WILD RELATIVES, CITRUS FRUITS, AND HYBRIDS OF THE GENUS CITRUS. *In* Jour. Agr. Research, v. 14, no. 9, p. 350. 1918.

barrier, despite its promising features of canker-resistance and extreme vigor.[6] The recent discovery, however, that cuttings of the citrangequat may be rooted readily by the use of new methods, has been so encouraging that extended trials of this plant as a stock are now under way. By the use of rooted cuttings, practically a full year's time may be saved in growing stocks to budding size, an advantage certainly sufficient to offset any additional trouble and expense for greenhouse equipment. A combination of bottom heat, about 90° F, with humidity maintained at a high point, 80° to 90°, and with air temperature lower than that of the soil, 70° to 80°, is essential to the best results.[7] With good control of all these factors, small twig cuttings with leaves attached may be rooted within a few weeks—in less time, in fact, than it usually takes for seed to germinate.

Plate 3 shows two of these cuttings and the original seedling plants beside them, with the stubs visible where the cuttings were removed. These cuttings were photographed only 4½ months from the time they were placed in the rooting bed and are fine specimens of a large lot rooted in February, 1921. The seedlings were 18 months old. An interesting point in this connection is the fact that while the citrangequat roots so freely the three parent species utilized in creating the hybrid (the sweet orange, trifoliate orange, and kumquat) commonly fail to root under the same conditions.

By the use of rooted cuttings, certain advantages are secured well worth consideration. First, the time saved has already been mentioned; second, through vegetative propagation uniformity of vigor is secured which is highly desirable and cannot ordinarily be expected through the use of seedlings; and, third, it has often been noted in the propagation of cross-bred plants that there is a vigor of growth in the first generation seedlings not so apparent in succeeding generations of self-pollinated plants. This vigor can be utilized to best advantage by the rooting of cuttings from especially vigorous individuals.

Buds of Satsuma and other citrous fruits grow readily on citrangequat stocks, but tests under field conditions will be necessary before definite conclusions can be drawn. While such tests have been under way now for several years, more time will be necessary in order to secure trustworthy conclusions as to the effect of the stock on the quality, size, and season of fruit crops, as well as hardiness and other factors of practical importance.

Regardless of the possible utilization of the Thomasville citrangequat as a stock, its obvious value as a hardy, canker-resistant fruit tree for home use makes it desirable to give it a distinctive name and publish a detailed description.

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[6] The character of this hybrid as regards seed content seems to be dependent largely on the possibility of its receiving pollen from other seedy varieties of Citrus. In many places where grown by itself or along with seedless varieties (such as Satsuma, having little or no viable pollen) the fruit is practically seedless, while in other plantings where mixed with seedy varieties, seeds are usually found averaging six or eight to the fruit.

The Thomasville citrangequat, like many other hybrids between widely differing species of the Citrus group, reproduces itself unchanged from seed. This means that it is in reality a sterile hybrid and the embryo found in the seed is developed from the tissues of the mother plant and of course shows no effect of the pollen used in fertilizing the flower.

[7] The method used was developed in January and February, 1921, by Mr. Eugene May, jr., plant propagator of the Office of Crop Physiology and Breeding Investigations, and is an adaptation of the rooting methods devised by Mr. Edward Goucher, of the Office of Foreign Seed and Plant Introduction, Bureau of Plant Industry, United States Department of Agriculture, for the rooting of small twig cuttings of the Lychee. (GOUCHER, Edward. ROOTING LYCHEE CUTTINGS BY MEANS OF A HIGH TEMPERATURE AND HIGH HUMIDITY PROCESS. *In* GROFF, George Weidman. THE LYCHEE AND LUNGAN, Appendix 8, p. 157-159. New York, London, and Canton, China, 1921).

TECHNICAL DESCRIPTION OF THE THOMASVILLE CITRANGEQUAT

Fruit oval or nearly spherical, mature fruits having a long diameter of 1¾ to 2 inches, diameter in cross section 1½ to 1⅞ inches, calyx strongly persistent with usually 5 long, clawlike points; fruit spur long, swollen, and characteristically bent downward apparently by the weight of the fruit, color of mature fruit yellow to orange yellow,[8] rind thin (⅛ inch) somewhat roughened or "pebbly," oil cells small, depressed, segments, usually 7 or 8, thin-walled; core, little or none; seeds varying from none to 12, usually not more than 6, small and plump; pulp light greenish to amber color, juicy, pleasantly acid when immature, becoming sweetish when fully mature; tree evergreen, vigorous, of upright habit; thorns rather numerous on old wood but much reduced and inconspicuous on bearing twigs; leaves uni-, bi-, and trifoliate (on young plants chiefly trifoliate, on bearing wood chiefly unifoliate) long pointed oval or lanceolate, 2 to 3 inches in length, rather thick and stiff, slightly folded on the midrib, dark green, with finely incised margins; petioles, very narrow-winged; flowers small, pure white, 5-petaled, bloom appearing rather late though not as late as the kumquat, fruits ripening from October to December, but juicy and serviceable as ade fruits from July to October.

OTHER CITRANGEQUATS

During the spring and early summer of 1909, the senior author made many cross pollinations between citranges and kumquats. From these numerous crosses, 67 citrangequats were secured. These citrangequats are mostly hybrids of the oval kumquat with the citrange, the latter being the pollen parent. Five different citranges were used as pollen parents, but most of the citrangequats (49 in all) have Rusk citrange as the mother parent; the next largest group (11 in all) are hybrids between the Willits citrange and the oval kumquat. Three of the citrangequats are hybrids of the round kumquat pollinated by the Rusk citrange.

Not all of the citrangequats have been as yet carefully studied, but a number of them are of interest and several of the most promising are noted below.

A second citrangequat (C. P. B. No. 48011) was grown from the same crossed fruit that yielded the Thomasville. It differs from that variety in having large fruits more nearly spherical in shape and borne on upright fruit stalks instead of on curiously elongated "goose-neck" fruit stalks. This citrangequat, like the Thomasville, loses much of its acidity as the fruit matures and like it has a mild-flavored peel and usually few seeds. When fully ripe this citrangequat is sweeter than the Thomasville and can be eaten from the hand by those who like rather sour citrous fruits.

Another citrangequat resulting from the same cross of oval kumquat and Willits citrange is C. P. B. No. 48005. The pollen parent was the same as that of the Thomasville but the seed parent was a different tree growing in the same dooryard at Eustis, Fla. This citrangequat has attracted attention by fruiting freely in the garden of Mr. George H. Harris, a cooperator living at McRae, Telfair County, Ga., far to the north of the limit of ordinary citrous fruit culture.

It differs decidedly from the Thomasville in remaining intensely acid even when fully mature. The fruits are nearly spherical and are borne on erect fruit stalks. As this citrangequat has fruited successfully as far north as the latitude of Savannah, 32°, it seems advisable to give it a

[8] RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. pl. III, 15. Washington, D.C., 1912.

name, and it is accordingly proposed to call it the Telfair citrangequat (Pl. 2, C).[9]

Another citrangequat of the same parentage is C. P. B. No. 48007, which was conspicuous for its extreme vigor when young. (Pl. 1.) The fruit, like that of the Thomasville, acquires something of an orange flavor as it ripens. It seems to be an early ripening variety. The peel is mild flavored, and the seeds are few.

Still another citrangequat is C. P. B. No. 48050, a hybrid of the oval kumquat with the Rusk citrange. It attracted the attention of Mr. J. W. Benson, a cooperator living at Sinton, San Patricio County, Tex., near Corpus Christi, by its superior cold resistance and nearly seedless fruits. A tree that flowered in October, 1918, carried a crop through the winter unharmed. Samples received at Washington on April 23, 1919, were very like a lime in appearance and flavor, but showed a small, fleshy calyx, somewhat like that of the Rusk citrange parent. Fruits of this number, received November 1, 1920, from Glen St. Mary, Fla., were strikingly handsome, being bright orange or even flame scarlet in color. They were from 1½ to 1⅞ inches in diameter, nearly spherical, and had few or no seeds. These fruits were overripe, but the pulp was still acid, so this is doubtless an early maturing variety suitable only for ade.

As this citrangequat has attracted attention, not only because of its nearly seedless lime-like fruits produced in Texas, but also because of the extraordinarily brilliant color of the fully ripe fruits in Florida, it seems best to give it a name. It is accordingly called the Sinton citrangequat.[10]

EUSTIS LIMEQUAT

In 1909, the senior author originated a new type of citrous fruits by crossing the West Indian lime with the kumquat oranges. Those familiar with citrous fruits know that the lime is the tenderest of all the commonly grown species of this group. It frequently freezes to the ground even in southern Florida, so that its culture is chiefly confined to the fringe of keys along the coast. The kumquat, on the other hand, is one of the hardiest of the evergreen citrous fruit trees. This is without doubt not so much due to its direct powers of cold resistance as to its remarkable dormancy. The kumquat, as noted above, is able to pass unaffected through long spells of hot weather that force other Citrus trees into a tender and succulent growth that is liable to be injured by even a light

[9] TECHNICAL DESCRIPTION OF THE TELFAIR CITRANGEQUAT: Fruit spherical (sometimes slightly flattened or elongated) mature fruits having a diameter of 1¼ inches to 1½ inches; calyx persistent with 5 long points; color of mature fruit light orange yellow to deep chrome (RIDGWAY, Robert, OP. CIT., pl. III, 17, b, d); rind thin, somewhat roughened; oil cells prominent, small, numerous; core little or none; segments 4 to 6 (usually 5), seeds few, variable in size, usually undeveloped, pulp pale yellow; Pinard yellow, (RIDGWAY, Robert, OP. CIT., pl. IV, 21, d.), sharply acid, and retaining this quality when mature; rind slightly bitter; tree evergreen, vigorous, of upright habit; leaves uni-, bi-, and trifoliate, generally obovate, 1½ to 2¼ inches in length, finely serrated; plant fairly thorny, thorns smaller in size on the fruiting twigs; bloom often occurring over a long period, giving a semieverbearing fruiting habit.

[10] TECHNICAL DESCRIPTION OF THE SINTON CITRANGEQUAT: Fruits varying from slightly flattened to slightly elongated; growing in clusters; diameter varying from 1½ inches to 1⅞ inches; calyx small, persistent, slightly protruding, sunken pistil surrounded by a very small raised circle; color variable, ranging from a light orange-yellow (RIDGWAY, Robert, OP. CIT., pl. III, 17, d), to a light flame scarlet (RIDGWAY, Robert, OP. CIT., pl. II, 9); rind very thin, smooth, highly colored, 1/16 inch in thickness and much like kumquat, showing almost none of the flavor of the trifoliate orange; oil cells few, variable in number; segments 7 to 8; no seed in 100 of fruits, occasionally one or a few; pulp pale, varying from orange-buff (RIDGWAY, Robert, OP. CIT., pl. III, 15, d), to a light cadmium (RIDGWAY, Robert, OP. CIT., pl. IV, 19); flavor sharply acid, decidedly lime-like with only faint suggestion of citrange flavor given by the oil from the rind, no citrange flavor in the juice; makes a good ade; core very small and segment walls very thin; tree much more hardy than the lime; bearing twigs spineless; leaves on bearing branches unifoliate, rather thin, 2 to 3 inches in length by 1¼ inches in width; petiole ⅛ inch long, slightly winged; midrib heavy, prominent on upper surface; leaves tapering slightly toward the apex.

frost. Some measure of the dormancy of the kumquats is indicated by the fact that they flower from two to three months later than other citrous trees.

This series of crosses between the lime and the kumquats was made at Eustis, Fla., in June, 1909, in the Citrus grove of Mr. F. W. Savage. These crosses resulted in a number of hybrids varying in character, but all having fruits much like the lime in quality. Attention has been attracted to one of these hybrids (C. P. B. No. 48798) that fruited during the summer of 1918 in the greenhouse of the Department of Agriculture at Washington, D. C. It resulted from fertilizing the flowers of the common or West Indian lime with pollen of the round kumquat.

The Eustis limequat fruit is of striking appearance, strongly resembling a West India lime in color, size, and texture. (Pl. 4.) The color is a light yellow, resembling the color of grapefruit. When cut, the fruit shows its lime-like character. It is very juicy, thin-skinned, has few seeds, and the flavor can hardly be distinguished even by an expert from the true lime. Moreover, the rind is edible, like that of the kumquat, so that the whole fruit may be utilized. It is particularly promising as a fruit for crystallizing. The spines on the bearing wood are very inconspicuous, a decided point in favor of this hybrid in contrast with the viciously spiny character of the common lime. Some of the other limequats exhibit rather long spines, even on the small twigs. Observations covering several seasons indicate that this limequat possesses much of the immunity to disease and insect pests enjoyed by the kumquat. The kumquat is the most resistant to Citrus-canker of all citrous varieties, a resistance amounting to practical immunity, an advantage the limequat has in some degree.

Specimens submitted to Citrus experts and growers have in every case elicited favorable opinions, it being held that the limequat's place in citrous fruit culture is not dependent entirely upon unusual hardiness. Even should it prove no more hardy than the sweet orange, it would still be of great value throughout the orange-growing regions, as the lime is so extremely tender that its culture is now almost entirely confined to the protected keys along the Florida coast. This hybrid, as well as several sister hybrids of this limequat, have been fruited at Glen St. Mary, Fla., in the northern tier of Florida counties, but were killed in the successive freezes of 1917 and 1918. With a little protection while young, and during unusually severe weather, it is probable that this hardy form of lime could be grown in the warmer parts of the Gulf Coast, supplying a real need in this region.

In Florida, California lemons are not obtainable owing to the quarantine restrictions necessary to prevent the introduction of "brown rot." Sicilian lemons are often expensive and are only obtainable in the larger towns, so that it often happens that a good acid citrous fruit for ade making is a decided scarcity even in Citrus-growing territory.

Budding experiments on different stocks are in progress and the present indications are that the limequat will thrive on most of the stocks commonly used except sour orange. Unlike the Thomasville citrangequat the Eustis limequat does not come true from seed. Nursery propagation is chiefly confined to the rough lemon and trifoliate orange stocks. Like the lime, if planted low and mounded with soil the limequat bud will strike root, thus simplifying the stock question. Its fruiting season is also of importance. To be of the greatest service in the home fruit garden it should bear the bulk of its crop in the warm summer months.

The indications are from the few trees that are in bearing in Florida that it will prove more or less everbearing, following the lime parent in this regard. No doubt its fruiting season can be influenced by methods of fertilization and pruning. Selection of budwood from early or summer-bearing trees will also be desirable.

The limequat promises to become a valuable addition to our list of citrous fruits. Since the original cross pollination resulting in this new fruit was made at Eustis, Fla., a well-known Citrus-producing center, it is proposed to name this hybrid the "Eustis limequat."

TECHNICAL DESCRIPTION OF EUSTIS LIMEQUAT

Fruit oval (occasionally nearly spherical) slightly asymmetrical, mature fruits having a long diameter averaging 1⅝ to 1⅞ inches, diameter in cross section, 1⅛ to 1½ inches; calyx persistent; color light yellow ("picric yellow")[11]; rind thin, $\frac{1}{16}$ inch, very smooth and glossy, translucent; oil cells spherical, rather prominent, having no bitter flavor; segments 6 to 9; seeds 5 to 12, usually averaging 1 to the segment, small to medium size, ¼ to ⅜ inch in length; pulp of light greenish color, closely resembling lime, tender and juicy, flavor sharply acid (like the lime the fruit can be used when full sized but not fully colored); tree evergreen, vigorous, having a tendency to produce long shoots which bend downward with the weight of the fruit; resembles the lime in producing a succession of crops, though not so markedly everbearing; spines on bearing twigs very small, not injuring the fruit; flowers 5-petaled, pure white (not streaked with pink as those of the lime and of most other limequats); leaves unifoliate, rather thick, dark green above, lighter below, tapering at both base and apex, 2 to 3 inches long, slightly folded along the midrib, having short, narrow petioles, closely resembling leaves of the round kumquat except for larger size.

OTHER LIMEQUATS

In addition to the Eustis limequat, some 28 other hybrids were made by the senior author in 1909 between the West India lime and the kumquat. Twenty-four of these, like the Eustis, are hybrids of the West India lime and the round kumquat; four are hybrids of the West India lime and the oval kumquat. Only about one-third of these have been studied in detail, but several of them are being grown in Florida and other Southern States. Perhaps the most promising of these is a limequat (C. P. B. 48786) of the same parentage as the Eustis; that is another cross between the same two parent trees. This is the largest limequat yet produced, sometimes equaling a small lemon in size, usually being about 1¾ inches in diameter and 2¼ inches long. The fruits, bright yellow when ripe, have a very pale pulp of a pleasant acid flavor, similar to the Mexican lime, the skin is very thin but tough and of a mild flavor. There are from two to nine seeds in a fruit.

This limequat has done particularly well in the garden of Hon. H. J. Drane at Lakeland, Fla., and as it is likely to be propagated more or less it seems desirable to give it a name. It is accordingly called the Lakeland limequat (Pl. 5, A).[12]

Another limequat that has attracted much favorable notice is C. P. B. No. 48792. This is a hybrid of the Mexican lime with the oval kumquat,

[11] RIDGWAY, Robert, OP. CIT., pl. IV, d 23.

[12] TECHNICAL DESCRIPTION OF LAKELAND LIMEQUAT: Fruits very smooth, attractive in appearance, oval, average 2¼ inches long 1$\frac{11}{16}$ inches in diameter; calyx not persistent; color Strontian yellow (RIDGWAY; Robert, OP. CIT., pl. XVI, 23); rind very thin, smooth, sweet and edible like that of kumquat; oil cells large and prominent, transparent, showing through the rind, segments 5 to 8, seeds 2 to 9, large and oval, usually about 6; pulp pale, massicot yellow (RIDGWAY, Robert, OP. CIT., pl. XVI, f, 21), resembling lime; very juicy, sharply acid, that of lime; good before fruit is fully colored; leaves 2½ to 3 inches in length; evergreen, unifoliate, lanceolate acuminate, petiole short, very narrowly winged; tree vigorous, bearing wood nearly spineless.

whereas the Eustis limequat and the Lakeland limequat are hybrids of the Mexican lime with the round kumquat. As this limequat has already been propagated to some extent in Florida and is certain to become more or less widespread as a dooryard fruit, it seems best to give it a name. It is accordingly called the Tavares limequat,[13] Tavares being the town adjoining Eustis, between which two cities lies Mr. Frank W. Savage's orange grove, where this and all the other limequats were produced.

The fruits are very similar to those of the Eustis limequat in appearance, and have much the same flavor. (Pl. 5, B.) It is often difficult to distinguish the fruits of these two varieties, the Tavares and the Eustis. On the whole the Eustis seems to be rather better in quality than the Tavares. The two can easily be distinguished by the fact that the flower buds of the Eustis are pure white while those of the Tavares have a pinkish coloration, like those of the West Indian lime.

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[13] TECHNICAL DESCRIPTION OF TAVARES LIMEQUAT: Fruits obovate or oval, occasionally nearly spherical; $1\frac{3}{4}$ to $1\frac{5}{16}$ inches long, $1\frac{1}{4}$ to $1\frac{7}{16}$ inches in diameter; light cadmium yellow (RIDGWAY, Robert, OP. CIT., Pl. IV, 19'); rind, very thin, smooth, tender, edible, averaging $\frac{1}{16}$ inch in thickness, mild flavored, stronger and tougher than that of kumquat and other limequats; segments 7 to 8; segment walls thin; seeds large, 6 to 11, usually 8 or 9, oval, often pointed at one end; pulp mustard yellow (RIDGWAY, Robert, OP. CIT., Pl. XVI, b 19'), resembling lime; juicy, sharply acid; leaves evergreen unifoliate, lanceolate; petioles very narrowly winged; tree vigorous with short spines on bearing wood; flower buds pink

PLATE 1

Four citrangequats and one oval kumquat, nearly 1 year old; nurse-grafted when 2 months old, on pummelo stock. From left to right C. P. B. No. 48005 (Telfair); No. 48007; No. 48010 (Thomasville); No. 48036; No. 48049 (oval kumquat). About $\frac{1}{10}$ natural size.

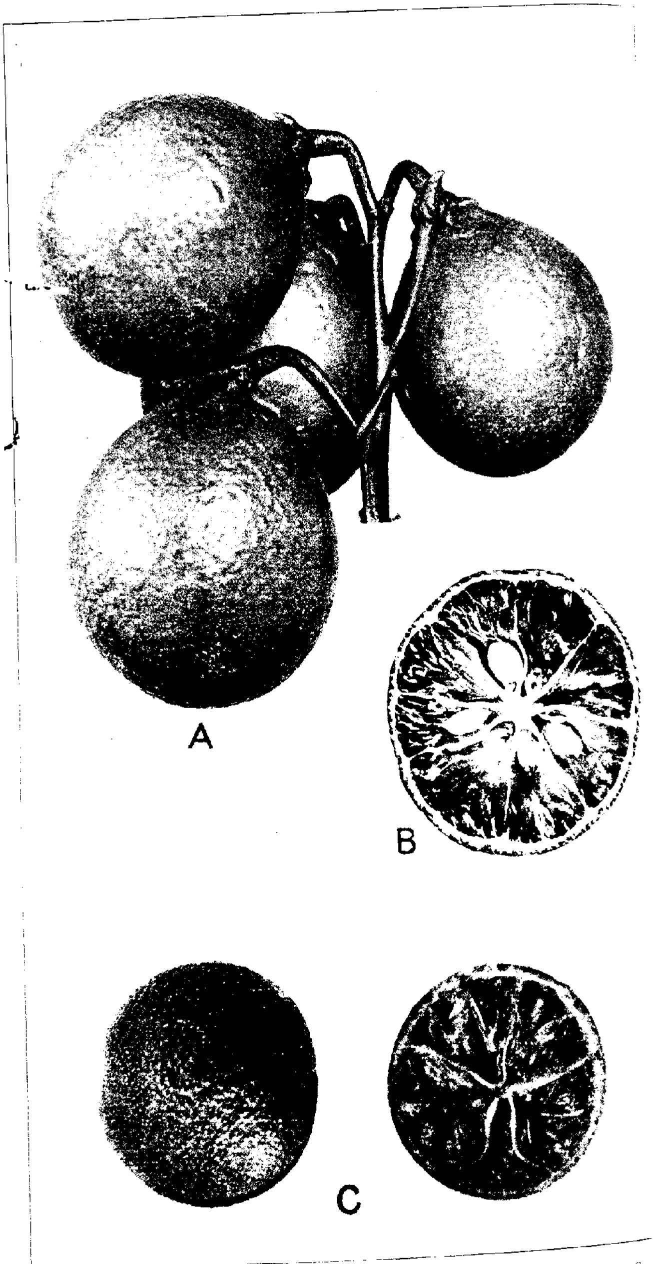
A
B
C

PLATE 2

A.—Thomasville citrangequat (No. 48010). Natural size.

B.—Cross section of Thomasville citrangequat sent in by Mr. A. M. Troyer to the Bureau of Plant Industry, from Fairhope, Ala., February 1, 1922.

C.—Telfair citrangequat (No. 48005) from McRae, Telfair Co., Ga., September 28, 1921. Natural size.

PLATE 3

Two Thomasville citrangequat cuttings (at left and right) each 4½ months old. In the middle two 18-months-old seedlings, showing the stubs from which the cuttings were taken. About ⅓ natural size.

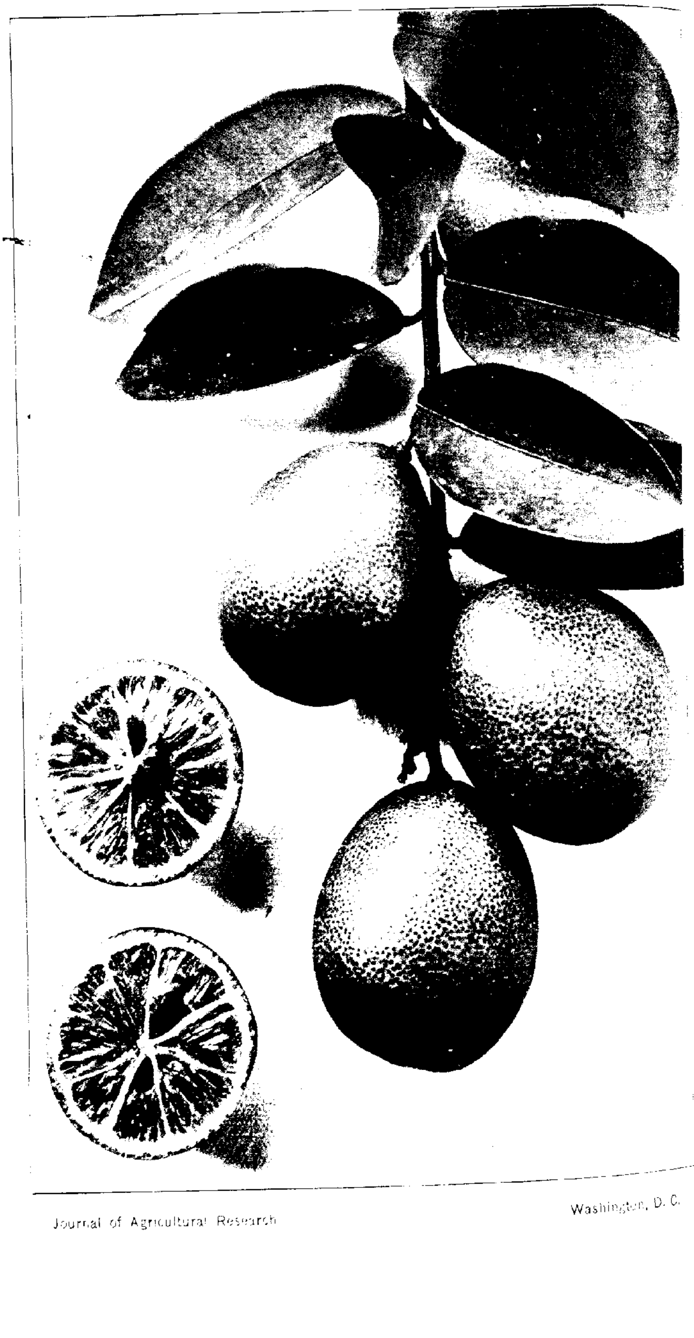

PLATE 4

Eustis limequat (No. 48798), grown in the greenhouse at Washington, D. C. Natural size.

PLATE 5

A.—Lakeland limequat from Lakeland, Fla., November 6, 1919.
B.—Tavares limequat from Glen St. Mary, Fla., December 23, 1921.


A
B

PRODUCTION AND DISPERSAL OF CONIDIA IN THE PHILIPPINE SCLEROSPORAS OF MAIZE [1]

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INTRODUCTION

The members of the genus Sclerospora have come to be recognized as among the most destructive of the downy mildews (Peronosporaceae). They long have been responsible for considerable injury to such cereals as millet and wheat in Europe and have recently occasioned alarming losses of maize and related crops in the Orient.

The genus is known to produce two types of spores; conidia which are not at all hardy but serve to spread the disease rapidly under favorable conditions, and resistant oospores which enable the fungus to persist through such unfavorable circumstances as drought, extremes of temperature, or freezing. Unfortunately, however, comparatively little has been known of the methods of spore production and dispersal in the case of these fungi, and it can even be said, as Butler (*3*),[3] who has contributed largely to our knowledge of the oriental forms, remarks:

> The life history of the cereal downy mildews, which all belong to the present genus, is the most obscure among the Peronosporaceae.

By the early European workers who described the type species (*Sclerospora graminicola* (Sacc.) Schroet.) on which the genus Sclerospora was founded, most of the emphasis was laid on the oospores, and very little attention was paid to the conidial stage, the meager descriptions and illustrations of this phase obviously depicting herbarium material unfavorable for study (*11*, p. 437–439, fig. 7). Recent investigation of the several downy mildews of the Orient, however, has of necessity dealt chiefly with the conidial stage, as in almost all of these cases the oospores apparently play little, if any, part in the life history. As a result, our appreciation of the importance of the conidial phase of Sclerospora has been increased, and our knowledge of the characteristics of the conidiophores and conidia has been augmented by further information.

Unfortunately, however, the available information concerning the conidial stage of the Sclerosporas is still all too scanty. For instance, no data have been furnished on the process of conidiophore and conidium production, its duration, its amount, and its relation to environmental conditions. Yet such information is obviously of primary importance, as spore production and dispersal are intimately related to the distribution and severity of the disease; and in a complete knowledge of all phases of these processes lies the means of intelligently effecting control.

During the past two years the writer studied the destructive downy mildews of maize which are common in the Philippines and found that

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[2] The writer wishes again to express his gratitude to Dean Baker, Prof. Reinking, and Prof. Elayda, of the Philippine College of Agriculture, for kindly supplying laboratory accommodations and other aid, and to Dr. Merrill, of the Philippine Bureau of Science, for generously extending the facilities of that institution.

[3] Reference is made by number (italic) to "Literature cited," p. 277–278.

two closely related but specifically distinct conidial Sclerosporas were causal there. One of these, *Sclerospora spontanea* Weston, occurs on maize and the wild grass *Saccharum spontaneum L.* in the Visayan Islands and is particularly distinguished by its long, slender conidia; while the other, *Sclerospora philippinensis* Weston, occurs on maize in the Island of Luzon and has relatively short conidia. Papers describing the characteristics and discussing the destructiveness, distribution, and relationship of these species already have been published (*24, 25*).

In the course of this work the process of conidium production and dispersal has been worked out; and as this phase of the life history of Sclerospora has never before been investigated in detail and shows several points which are of considerable importance in their relation to control, and of some interest from their mycological aspect, these are presented in the following paper.

PRODUCTION OF CONIDIA

As has been reported previously by the writer (*24, 25*), the conidial stage is the important one in the life history of the Philippine maize mildews, as it is by means of this stage exclusively that the widespread and serious destruction of maize is accomplished in the Philippine Islands, the oospores being as yet unknown on this host. The most vital phase of the conidial stage is the complex and hitherto obscure process of conidiophore and conidium production. Detailed consideration of this matter necessitates discussion of the following points: the demarcation of the conidiophore-bearing areas through preliminary paling of the infected tissue; the influence of environmental conditions, especially moisture, in inducing conidiophore production on these areas; and the emergence and development of the conidiophores thus induced. In addition, the nocturnal cycle of conidiophore development under various conditions, the long duration of this production in relation to the life of the host, and the magnitude of the numbers of conidia produced will be considered. Because the two causal species of Sclerospora agree essentially in all the main features of conidium production, the following account of the process except as noted applies equally to both.

PRELIMINARY PALING OF THE CONIDIOPHORE-BEARING AREAS

When a maize plant becomes infected with Sclerospora, it first develops on the normally green leaves markings of a more or less conspicuous pale yellowish white color (*Pl. 1 B*), and later it shows on the areas thus marked a down or felt of innumerable conidiophores and conidia (*Pl. 1, A*). These etiolated markings are always present in infected plants but vary greatly in extent, in shape, and in color according to the effect of such factors as the individual and varietal character of the host, the age at which it became infected, and the kind of climate and soil which have influenced it subsequently. As a result, it is not possible to cover the range of variation by any general description of the markings. To be sure, they may be grouped roughly into such categories as the types 1, 2, and 3 recognized by Palm (*16*) in the Javan maize mildew; but it should be remembered that these types are not differentiated with absolute distinctness, but intergrade.

Certain types of markings are not associated with conidiophore production and hence need not be considered here. Palm's third type, with slender golden striping decreasingly extensive on successive leaves, and the markings of similar characters found by the writer (*25*) to result from

infection through suckers, as well as the inconspicuously marbled markings described later in this paper as the result of preventing the access of dew or other moisture to infected leaves, are of this kind.

Certain markings, however, are always associated with conidiophore production. The most common of these have been described and illustrated by the writer (*24*) for the maize downy mildew of the Philippines, by Butler (*3*) for that of India, and by Palm (*16*) and Rutgers (*20*) for that of Java. The photographs, diagrams, and colored plates in these papers show the general characteristics and the range of variation in color and pattern of these markings. Consequently, no further description is necessary at this point.

It should be noted, however, that as a rule the markings associated with conidiophore production are progressively more extensive on successive leaves. This is especially noticeable in those markings which, because of their extremely pale color and large areas, are particularly conspicuous; and which, because they are associated with excessively abundant conidiophore production, are of especial concern to us here. In general, a leaf showing such marking has at the base a more or less extensive solidly discolored area from which irregular, jagged, tongued protrusions extend toward the tip. On the lowest leaf attacked (which may be any from the second or third up to the eighth or ninth) this discolored area is confined to a small portion of the base of the blade, while on each successive leaf the area increases in extent and the irregular protrusions run farther and farther progressively toward the tip, until on the last leaves the yellowish white discoloration occupies the whole blade (fig. 1).

The development of this pale color throughout these leaf markings of the diseased plant is a gradual one. When infected but as yet normal plants are kept under constant observation during the appearance of the disease symptoms, it is noticed that in the normal dark green of the leaves that are already unfolded, areas of the characteristic shapes that have been described, pale gradually through a yellowish green, then yellow, to a yellowish white color. The process is usually a gradual one, requiring several (6 to 24) hours from the beginning of the first difference in shade until the assumption of the final persistent whitish color. Since this loss of color is due to changes in the chlorophyl-bearing cells of the leaf brought about by the invading hyphae of the parasite, it is remarkable that so decided an alteration can be effected in what is relatively so short a time. The whitening of these areas may take place apparently at any hour, but in most cases observed it has been seen to occur during the night or in the early morning.

It should be emphasized particularly that the extent of these discolored areas is established at the beginning, and no increase in size takes place as time goes on. If, as soon as the discolored areas of an infected plant have paled sufficiently to be distinguished, they are outlined lightly with waterproof ink, it can easily be seen that when once defined they never grow out toward the tip of the leaf, but their outlines remain unchanged in extent. Moreover, if in a downy-mildewed plant one dissects out the young leaves, on the tips of which the markings can be seen as they unroll from the bud, the discolored areas on the inclosed part of the leaf will be found already faintly defined. These faintly distinguishable areas may, to be sure, expand somewhat as the unrolling leaf expands to its normal dimensions, but they show no progressive growth once the marked areas are established.

RELATIONSHIP OF CONIDIUM PRODUCTION TO ENVIRONMENTAL CONDITIONS

After the infected corn plant has developed the peculiar yellowish white markings which are characteristic of the disease, the production of conidiophores and conidia on these areas begins. Just how soon this can occur has not been determined; but in most of the cases observed, in

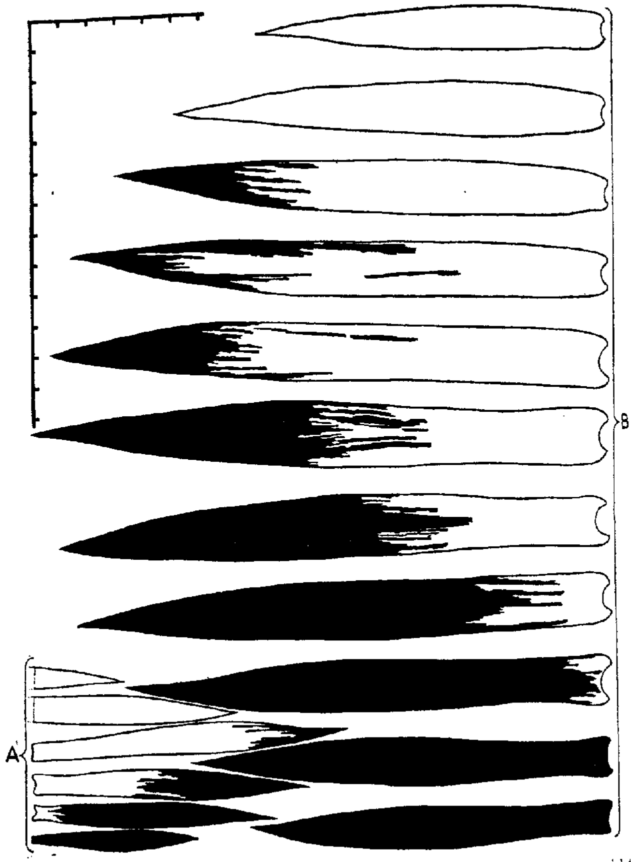

FIG. 1.—Diagrams (from tracings) showing the characteristic configuration and extent of the etiolated conidiophore-bearing areas on successive leaves of conspicuously diseased maize plants (A, plant 1; B, plant 10, Table II). The etiolated area (white) is increasingly greater and the unaffected area (black) increasingly smaller on successively developed leaves. The proportion of the total surface which produces conidia is relatively large. Scale in inches.

which the paling of the symptomatic markings took place at night or in the early morning, conidium production from these diseased areas began the next night if environmental conditions were immediately favorable. Under less favorable circumstances, however, several nights sometimes elapsed before conidia were produced.

The production of conidia from the diseased plant takes place almost without exception at night. Of the conditions which obtain during the night in the Philippines, the most essential to conidiophore production is apparently the presence of a layer of moisture on the surface of the infected plants. In this regard dew plays the foremost part under customary field conditions, while gentle rains are of somewhat less importance. The film of moisture must not be temporary but must persist for at least four or five hours to permit the conidiophore buds to protrude from the stomata, develop into conidiophores and mature conidia. The maximum production of conidia is attained when the whole plant surface is drenched and dripping with a heavy deposition of dew, as happens when still, clear, cool nights follow hot, rainy days. A gentle, drizzling rain persisting through the night and keeping the surface of the corn plant covered with a layer of moisture also results in conidium formation, though to a less extent. During violent, nocturnal storms, however, when the plants are beaten with driving torrents of rain, comparatively little conidium production occurs, and then only on the more protected parts of the plant where the delicate conidiophores can develop uninjured. No conidium formation takes place on nights when there is no deposition of dew on the infected plants, or when the temporary surface layer of moisture, whether of dew or rain, is dried in a short time by inopportune winds.

That the nocturnal production of conidia by the downy mildew depends on the presence of a layer of moisture over the host leaves was found to hold true not only under natural conditions in the field but also under the controlled conditions of various experiments. A number of maize seedlings, after being inoculated with the downy mildew, were divided into two lots, one of which was exposed to the usual field conditions, while the other was kept indoors protected from dew, rain, or other surface moisture. After the normal incubation period both lots developed the discolored markings characteristic of the disease; but although on successive moist nights the exposed plants produced abundant conidiophores, the plants indoors, on the contrary, consistently failed to produce any conidiophores whatever. After this had gone on for 10 days a few plants from the exposed lot were brought indoors, and a like number of the indoor lot were placed in the field. On the first favorably moist night thereafter conidiophore production took place on the latter newly exposed plants, although previously, while indoors, this had never occurred. On the contrary, the plants which had been supporting abundant conidiophore production in the field now, when brought indoors, ceased to bear any conidiophores whatever. Those other plants of the original lots which had been left indoors or had remained in the field continued to behave as heretofore. By varying the procedure in a series of experiments such as this, the dependence of conidiophore production on nocturnal moisture on the host was clearly demonstrated. When once this dependence was realized, it was comparatively simple to manipulate conditions so that the formation of conidiophores was induced or stopped at will.

It should be noted, however, that conidiophore production on an infected plant can not thus be induced month after month indefinitely, but if continued at intervals in experiments will gradually cease, being limited in duration as it is in plants growing undisturbed in the field. For example, if under field conditions the production of conidia

on a set of plants extends over two months, then this time will be the limit for inducing conidium formation on a similar set of plants which after inoculation have been kept indoors except when exposed to dew for occasional tests. It should be noted also that the markings of the disease on inoculated plants indoors are relatively inconspicuous when they appear and gradually become less and less marked until eventually they are so greenish in color and so broken up and interrupted in pattern that they are only recognized with difficulty.

In general, conidium production by the downy mildew takes place from the surface of the host chiefly on the discolored areas characteristic of the disease (Pl. 4, B, C, D). It may also occur, although less abundantly, on areas of the host that are apparently normal. Microscopic examination shows that the discolored areas harbor the most abundant mycelium in their tissues; but even areas that are seemingly healthy, and in fact any part of the diseased plant except the roots, may be invaded to some extent by the Sclerospora hyphae. As a result, in the case of plants which have become infected after they have attained sufficient growth so that they can struggle along to maturity in spite of the parasite, abundant conidiophore production may occur not only from the leaves but also from the leaf-sheaths (Pl. 4, E), from the husks Pl. 5, B), from the abnormal bract-like structures which are produced by diseased tassels and ears (Pl. 5, A), from the upper internodes of the stem, from normal parts of the tassel, and from the green, exposed tips of infected ears.

Conidiophore production may take place on both surfaces of the leaf. Whether the conidiophores emerge from the upper or lower surface of a leaf depends first, on the presence of stomata through which the conidiophores may develop, and, second, on the presence of a sufficient layer of moisture to induce this development. In maize the distribution of the stomata is rather uniform, the number in the lower surface of the leaf exceeding only slightly those in the upper. As a result, if other conditions are equal, the abundance of conidiophore production may be somewhat greater from the lower surface, but as a rule it appears to be about the same on both.

The presence of a layer of moisture on the leaf surfaces is determined by climatic conditions. During a gentle, drizzling, nocturnal rain there is deposited on the exposed upper surface of the leaf a sufficient layer of moisture to induce conidium formation there, while the protected lower surface is for the most part dry and bears no conidiophores. Dew, however, is usually deposited rather uniformly over the infected plant, on both the upper and lower surfaces of the leaves, and hence induces conidiophore production which either is approximately equal on both surfaces or else is somewhat more abundant on the lower, because of the more numerous stomata there.

This relatively even distribution of the conidiophores induced by dew is the type most frequently encountered on maize in the Philippines, and the reports of Butler (*3*), Rutgers (*20*), and Palm (*16*) that conidiophores occur on both surfaces but are slightly more abundant on the lower would indicate a similar condition in the case of the other oriental maize mildews. On sugar cane, however, at least on the only conidiophore-producing plant which the writer found in the Philippines, the conidiophores are almost wholly confined to the under surface of the leaves, and this is true also of the wild grass *Saccharum spontaneum*.

That this is not due to any character inherent in the fungus is shown by the fact that both species of conidial Philippine Sclerosporas behave in this way when growing on *S. spontaneum* and yet develop conidiophores on both sides of the leaves when growing on maize. On the contrary, the restriction to the lower surface of the leaves of sugar cane and the related wild Saccharum is attributable to the fact that in this genus by far the greater proportion of stomata are on the under surface. Dickhoff (*6*) and others have found this condition in sugar cane, and the writer has corroborated it in the wild Saccharum. It is of interest to note also that Miyake (*15*) reports that the Formosan downy mildew forms conidiophores only on the under surface of the leaf in sugar cane but on both surfaces in maize and teosinte.

When the production of conidiophores and conidia by the Sclerospora mycelium in a leaf first begins, it is not usually abundant. It starts on the first night with the protrusion of a few scattered conidiophores from the surface of a badly affected area usually along or adjacent to the midrib. During succeeding nights more and more conidiophores appear until finally vast numbers are produced nightly. As a rule, conidiophores appear first on the lowest leaves and leaf sheaths, which are markedly affected, and later develop progressively upward on the younger leaves of the plant.

After discovering that conidiophores were produced by the downy mildew only at night, the writer naturally endeavored to determine what factors operative during the night served to induce the mycelium of the fungus to send out conidiophores through the stomata of the host leaves. The conditions of the environment which prevail at night are darkness, lowered temperature, and the deposition of a layer of moisture on the surface of the host plant; the plant's stomata are nearly or quite closed, and metabolism is altered after the cessation of the photosynthesis which went on during the day. Hence, changes follow in the chemical content of the host cells and even of the gases in the substomatal chambers.

An attempt was made to determine by experiment which of these factors, or combination of interacting factors, was involved. It was found that darkness was not operative alone, nor in conjunction with the closed stomata and the metabolic changes following cessation of photosynthesis. No conidia were produced during the day time on infected plants kept in dark chambers, either at normal temperatures or cooled by ice; nor at night on plants which were cooled naturally and which had been undergoing normal photosynthesis in the sun during the day. Hence, it seems logical to conclude that neither darkness alone, nor darkness and lowered temperature combined, nor darkness and lowered temperature following normal exposure to the sun were the essential factors involved. Because in the first two instances the stomata were closed in plants in the dark chambers, and because in the third case the stomata were closed and the condition of the cells was the normal one after photosynthesis had ceased, it seems doubtful if these factors also are of any particular influence in conidiophore production.

The remaining factor, however, the presence of a layer of moisture deposited on the surface of the infected plant, does seem to be directly connected with conidiophore production. The writer found by a number of experiments, some of which have already been described earlier in this paper, that apparently it is the dew or other moisture on the surface of

the plants which induces the nocturnal conidiophore production. Just how this layer of moisture operates in this connection is unknown. Possibly the layer of moisture is effective by sealing the stomata and influencing the gas or moisture content within the substomatal chambers, so that this in turn affects the branches of the mycelium located there. It seems more probable to the writer, however, that this layer of moisture covering the stomatal pores furnishes the emerging conidiophores of the Sclerospora the necessary moisture for their development and that this development can not take place in the air alone. If this is the case, it arouses the interesting conjecture that this relationship may be of phylogenetic significance, an indication of steps by which the terrestrial Peronosporaceæ have been evolved from aquatic saprolegniaceous ancestors.

However, attempts to induce conidiophore production during the day by supplying a layer of surface moisture to the infected plants were entirely unsuccessful. Plants which had been actively producing conidiophores night after night were put in an ice chest and kept at a lower temperature for some hours; but although moisture was deposited on the inner walls of the container, the leaves, probably because their respiration prevented them from reaching the dewpoint, remained free from dew. The surface of the infected plant was, therefore, kept covered with a thin layer of moisture by repeated spraying. No conidiophores, however, were formed, although a darkened ice chest and also a light double-walled glass chamber maintained at the same temperature were used in the experiments. Moreover, heavily infected leaves of such plants when fastened so that they were submerged in jars of pure water produced no conidiophores either during the day or at night. These experiments are admittedly crude, and it is probable that the processes resulting in conidiophore production in the surface layer of moisture are delicate physiologic ones which will require careful study under the most exactly controlled experimental conditions before they are thoroughly understood. Eventually this phase of the problem should be carefully worked out; but at present, even though the relationship is not entirely understood, it can be said that the formation of conidiophores in both species of Philippine Sclerosporas—whether on maize, teosinte, sugar cane, sorghum, *Saccharum spontaneum*, or *Miscanthus japonicus* Anderss.—never takes place except when the surface of the host is covered with a persistent layer of moisture during the night or very early morning.

As condidiophore production in these Philippine Sclerosporas has been found by the writer to be restricted so rigidly to the night time, it is of interest to note whether any such restriction has been observed by investigators of the other important conidial Sclerosporas of the Orient. Apparently Miyake (*15*) alone appreciated that conidium production was nocturnal. Not only Butler (*3*) in his investigation of *Sclerospora maydis* (Rac.) Butl., in India, but also Raciborski (*19*), Rutgers (*20*) and Palm (*16*) in their successive studies of *S. Javanica* Palm, of maize in Java, failed to grasp this point. To be sure, Rutgers realized that there was a relationship between conidium production and moisture, since he stated that the fungus in the early morning was like a white down on the moist leaves while later in the day when the conidiophores and conidia were dried it gave a fine granular effect as of dried salt solution. Palm (*16*), on the other hand, regarded moisture as essential, not for the production, but for the germination, of the conidia, and consi-

dered it necessary to secure inoculating material early in the morning (around 9 o'clock) when the leaves were moist. Miyake (*15*) however, in his investigation of *S. sacchari* Miy., of sugar cane in Formosa, learned that conditions obtaining at night were in some way involved in inducing conidiophore production. In an endeavor to secure material suitable for germination experiments he placed infected cane leaves in glass jars which were inverted over shallow dishes of water. Under these conditions he found that deposition of spores always took place at night and never in the day time, no matter at what time these cultures were started. To observe the procedure more carefully he prepared similar moist chamber cultures which he examined hourly from 5 p. m. Deposition of conidia began at 1.30 a. m. and continued until 2.30 a. m. He concluded that during the day time the fungus prepares for conidiophore and spore formation which takes place during the night, but he believed his investigations needed repetition. Miyake realized that conidia were deposited at night under these laboratory conditions and inferred that this was true in the field also, but apparently he did not appreciate that this occurs repeatedly night after night on infected plants, nor did he attempt to determine whether moisture, darkness, or other night conditions are responsible.

DEVELOPMENT OF THE CONIDIOPHORES

Whenever and wherever it may occur, however, the development of the conidiophores and conidia follows the same general procedure. As has been said above, it is apparently the covering of the surface of the maize plant with moisture which influences the Sclerospora mycelium within the tissues of the host to produce conidiophores and conidia. After the host surface is thus covered, the stout mycelial branches which are located in the intercellular spaces and in the air chambers beneath the stomata (Pl. 7, G, H) begin to send out prolongations, several of which push through each partly closed stomatal pore (Pl. 7, B, C, D) and give rise to a compact group of several simple or branched knob-like processes (Pl. 7, E) which are closely appressed to the outer leaf surface, covering the stoma. From each of these knobs develops a clavate hypha (Pl. 8, B, C), the main axis of the conidiophore, which increases rapidly in size (Pl. 8, D, E) and finally forms at its apex the papillate buds (Pl. 8, J), which grow into the stout primary branches (Pl. 8, K, M). As these extend they, in turn, form at their apices the paired protrusions which become the secondary branches (Pl. 8, N); and in like manner the tertiary or even quarternary series of branches are developed (Pl. 8, O) until finally from the ultimate tips arise the sterigmata. From the apex of each sterigma buds out a conidium (Pl. 8, Q) which grows rapidly, beginning as a small, globular protrusion (Pl. 8, R, S) and passing successively in its development through globular pyriform, oval or ellipsoidal stages (Pl. 9, A–I), until finally it attains the size and shape, usually rounded cylindric, of maturity, and is cut off from the sterigma by a wall (Pl. 9, J, K, W).

These successive stages of conidiophore development require several hours for their completion. The formation of the group of knoblike conidiophore initials is not completed until the surface of the plant has been covered with moisture for two to four hours; and the development of the conidiophore and conidia to maturity consumes about three hours

more. The process is, however, a continuing one, the knoblike conidiophore initials developing into conidiophores one after another in fairly regular succession so that several stages of the transition from the initials to the completed conidiophore are usually found over a stoma (Pl. 7, F; 10, G, H).

In many cases the first "crop" of conidiophores which matures seems to be the maximum one, successive "crops" being somewhat less heavy. When badly infected leaves are examined at the time of maximum conidium production (usually about 2.30 a. m.) they are seen to present a striking appearance. The moist surface is covered with a dense grayish white "down" of innumerable conidiophores whose treelike shape can be distinguished on closer inspection.

Attempts were made to determine whether the groups of knoblike processes over the stomatal pores were renewed each night by growth from the internal mycelium, or were able, if not entirely used up in developing conidiophores during one night, to survive the day and form conidiophores the night following. In occasional instances, especially in *Saccharum spontaneum* with its sunken, protected stomata (Pl. 7, H), and during rainy or cloudy days, leaves sectioned during the day showed groups of knobs that were apparently still viable. As a rule, however, indications were that these groups dried up and were replaced by new ones which pushed out of the stoma on the next favorable night. Further investigation is necessary to settle this point conclusively.

It is noteworthy that the tips of the conidiophores customarily protrude slightly from the surface of their layer of moisture on the leaf, so that the conidia and sterigmata and perhaps even the ultimate branch tips are free, although beaded with adherent droplets of moisture or even covered by a single larger drop which involves the whole head. To some extent there is apparently a relation between the thickness of the moisture layer on the leaf surface and the height of the conidiophores. In scanty dew when the layer of moisture on the leaf is very thin, the conidiophores are short and stocky; while those which develop in deeper layers of heavy dew are usually longer. In both cases the conidia are borne just above the surface of the moisture layer. It should be noted, however, that the specific distinctions of length which exist between the conidiophores of *Sclerospora philippinensis* and *S. spontanea* are not invalidated thereby; since especially in their longer and more slender foot cells the conidiophores of the latter maintain their proportionate differences even when the two are grown under the same conditions (Pl. 8, O, P; 10, B, F).

DISCHARGE OF THE CONIDIA

In view of the fact that the conidia were borne just barely above the surface of the layer of moisture on the leaf, or even involved in adherent droplets, and yet (as will be shown later) were distributed in vast numbers by the slightest air currents, it did not seem possible to the writer that mere passive adjunction of the conidia from the sterigmata could be accountable for all that was observed. When, for instance, Petri dishes containing a thin layer of nutrient agar were placed at the base of a young, infected, corn plant from which abundant conidium production took place nightly, and when the whole was covered by a large bell jar firmly pressed into the earth and allowed to remain overnight, in the morning the surface of the agar was found covered with innumer-

able conidia. If the conidia merely dropped off the sterigmata, they would of course fall down to the Petri dishes from the lower surface of the more or less horizontal leaves; but those produced on the upper surface would hardly be carried off by the slight convection currents within the jar rapidly enough to prevent most of them from falling into the moisture on the upper surface of the leaf. Microscopic examination of the moisture on the upper and lower surfaces of the horizontal portion of such a leaf, however, showed no more spores in the former than in the latter, and relatively very few in either. Moreover, a whitish deposit of innumerable conidia often was observed on healthy leaves which, in crowded plantings, stood in close juxtaposition to infected, spore-bearing leaves. This occurred even when the parts were sheltered and were in such a vertical or oblique position as to preclude the possibility of such a deposit accumulating from conidia which merely fell.

From such observations the writer was led to suspect that the conidia were actively discharged. To obtain further information on this point the following experiments were performed on maize infected with *Sclerospora philippinensis*. During the afternoon the leaf of a badly diseased plant that was known to be producing abundant conidia each night was carefully scraped with a scalpel until its surface was entirely free from all dried remnants of the last night's conidiophores. The leaf was then fastened to a stake so that this scraped surface was held rigidly in a vertical plane. Facing this surface a slide, previously smeared lightly with clear agar, was fastened securely at a very slight angle, with the upper end almost touching the leaf and the lower end about 3 mm. away. The earth around the plant was then watered copiously; and the whole was covered with a large tin can the edges of which were pressed firmly into the earth and sealed with wet clay. Early the next morning this cover was removed carefully so as not to disturb the plant; and the slide was dexterously lifted away without touching it to the leaf surface. On examination the slide was found to be covered with freshly germinating conidia for some distance from the edge nearest the leaf. By substituting a strip of black cardboard for the slide, a faint spore-print, decreasingly dense toward the end farthest from the leaf, was obtained.

More direct evidence was secured as follows. Under a strong beam of light concentrated by a condenser, a portion of the leaf surface where abundant conidium production was taking place was watched continuously through low power magnification. Under these conditions the conidiophores, their tops heavy with clumps of maturing conidia, could be seen standing in rows corresponding to the rows of stomata below. During long observation occasional clumps of conidia were seen to flash away as momentarily gleaming specks, leaving a gap in the line of conidiophores.

In the opinion of the writer these experiments and observations indicate that the conidia are forcibly thrown off from the sterigmata. It will rest with future and more precise investigations to prove this conclusively.

Attempts were made to determine the means by which discharge of the conidia was accomplished. Because all efforts to grow the Sclerosporas in pure culture failed, it was impossible to study the developing conidiophores in Van Tieghem cells; and it was found equally impossible to adjust conidiophore-bearing leaves for study under the high power without disturbing the moisture on the leaf and interfering with conidium

discharge. However, in so far as could be determined by a study of conidiophores mounted in all stages before and after discharge of the conidia, the process apparently takes place as follows: At the point where the maturing conidium is attached to the sterigma, the wall across the base of the conidium and that across the apex of the sterigma are, when first formed, quite flat in contact with each other. Later the increasing turgidity of the conidium and sterigma causes these membranes to bulge outward, a tendency which is restrained by the adhesion of the two surfaces to each other. As this impulse to bulge outward increases more and more, however, it finally reaches a point where it overcomes the adhesion of the two surfaces; and with a sudden snap the basal septum of the conidium and the apical septum of the sterigma bulge sharply outward and shoot the conidium away. In abundant fresh material all the stages of this process can be traced, the flatness of the base wall of the conidium and the apex of the sterigma before (Pl. 9, L–V), and the bulging after discharge, being particularly noticeable.

Apparently all the conidia on one conidiophore customarily are ejected at once; but occasionally a conidiophore was encountered with most of its sterigma tips bulged out and obviously just freed from their conidia; while on one or two branches seemingly less mature, the conidia still remained in situ (Pl. 9, W).

When the conidiophore has discharged its conidia, its vitality declines, it is attacked by bacteria which swarm in the moisture on the leaf surface, and after dawn it is shrivelled by the sun to a dry, almost unrecognizable mummy (Pl. 10, C, D, E, H). In no case was seen any evidence, such as sterigmata with their apices burst open, which would indicate that an explosive ejection of the conidia, with loss of contents and subsequent collapse of the conidiophores, had taken place.

The discharge of conidia by the sudden bulging out of walls formerly flat in contact with each other was first described according to Buller (2) by Nowakowski for the Entomophthoreae. It has since been found by Buller (2) to take place in the case of the basidiospores of certain Hymenomycetes, and by Coons (5) also in the case of the sporidia of Gymnosporangium. In view of this, it is of interest to note that a similar method of spore discharge apparently obtains in the genus Sclerospora of a family (Peronosporaceae) in which the passive abjunction of the conidia has been assumed hitherto.

The distance which the Sclerospora conidia are ejected is very minute. With the apparatus available no accurate measurements could be made; but by such crude devices as the slanting slide mentioned above, the trajectory limit was approximately determined as about 1 to 2 mm. Small as this distance is, however, it suffices to carry the conidia into the air where they are wafted away by more or less rapid air currents; and it is noteworthy that this process of conidium discharge is remarkably well adapted to secure the dispersal by air currents of conidia flung from the moist surface of the maize plant.

NOCTURNAL CYCLE OF CONIDIOPHORE PRODUCTION

The process of conidium production which has been described follows a regular cycle under normal conditions of dew deposition. The leaves are wet with dew at 8 or 9 p. m. Groups of knoblike initials are formed over the stomata at about 11 p. m. and most of the first "crop" of conidia

has matured and is being discharged at 2 a. m. It should be emphasized, however, that this process is a continuing one; for during this time other conidiophores have been developing from the initials, so that from thenceforward a constant succession of conidia is developed and discharged. Although the maximum discharge of conidia usually takes place from 2 to 3 a. m. and lessens somewhat after that time, it does not entirely cease until the necessary moisture is dried by the early sun. This schedule applies consistently under the condition of uninterrupted dew which usually obtains during clear nights in the Philippines whether in dry, wet, or transitional seasons.

During rains, however, this schedule may be altered. When gentle rain begins in the afternoon and continues so that the plants have already been wet two or three hours by the time dew deposition begins, the formation of conidia takes place much earlier, beginning even as soon as 9 o'clock, reaching its maximum around midnight, and continuing under favorable conditions till dawn.

Observations made on May 24, 1919, furnish a good example of such a schedule. After a hot, humid day with a maximum temperature of 93°F., rain began at 4 p. m. and fell steadily for more than an hour. The sky remained clouded and the air, which was full of moisture, cooled rapidly, reaching 75° shortly after sunset and causing an early and heavy deposition of dew. At 8 p. m., when their surface had been moist uninterruptedly for 4 hours, infected maize plants were inspected. Microscopic examination showed that conidiophore production was indeed beginning, the conidiophores being in the earliest stages of development as club-shaped initials. At 9, development of the conidiophores had progressed further and the whitish down was visible under a lens. At 10, heavy conidiophore production was apparent, and quantities of conidia were matured. This production reached its maximum from about 11 until midnight but still continued, although less abundantly, at 5 the next morning, when the sun was just appearing. On plants protected from the early sun conidia were formed for about an hour more before drying. Production of mature conidia in this case continued for more than 8 hours instead of the 4 or 5 of the usual schedule.

When, after a night of abundant dew, the sky clouds over and a gentle rain begins at dawn, so that the necessary moist condition of the leaves is maintained into the morning by a constant drizzle, the process of conidium production may continue for a few hours longer. Similarly when maize is located in deep valleys or protected from the first rays of the sun by the shade of trees or hills, the dew on the infected leaves and the consequent production of conidia persists after the process has ceased on the already dried leaves of less sheltered plants.

During the dry season, also, the usual schedule may be altered. Occasionally conditions of temperature and moisture are such that dew deposition is very light and fleeting, and conidiophore production, beginning tardily, is scanty, restricted to moister parts of the plant surface and soon ceases. For example, on April 23, 1919, one of several successive dry, partly cloudy days, although the temperature, which had been 92° F. about noon, dropped gradually to 75° during the night, dew deposition was very late and scanty, as the sky was clouded and a warm, dry breeze blew fitfully. Frequent examination of infected maize plants showed that a very light dew began to gather at 10 p. m. At 3 a. m., in a thin film of dew on the leaves, a scanty production of

conidiophores was apparent. Microscopic examination showed that the first "crop" of conidia was maturing but that the conidiophores were dwarfed and scantily branched. A little after 4 a. m. conidiophore production was terminated when the dew dried as the breeze increased. In this case formation and liberation of conidia lasted for about an hour only.

The entire process of conidium production from the emergence of the internal hyphae through the stomata to the final ejection of the mature conidia takes place with remarkable regularity. Whether on infected maize, teosinte, sorghum, sugar cane, *Saccharum spontaneum*, or *Miscanthus japonicus*, whether on young seedlings or senescent plants, whether on leaves, tassels, bracts, or other infected parts, the cycle of conidium production under favorable climatic conditions follows a relatively constant schedule, which may be outlined as follows:

7 to 8 p. m.	Clear, still evening; dew deposition begins.
10 to 11 p. m.	Plant surface wet with dew; groups of knoblike conidiophore initials are forming over the stomatal pores on the leaf surface.
11 to 12 p. m.	From the knoblike bases the clavate main axes are developing and beginning to form primary branches of the conidiophores.
12 p. m. to 1 a. m.	The branch system of the conidiophore is now completely developed, and conidia are beginning to bud out, while the basal cell is being cut off by a cross wall.
1 to 2 a. m.	Discharge of the conidia and their dispersal by air currents begins to take place.
2 to 3 a. m.	Discharge and dispersal of conidia is at its maximum.
3 to 4 a. m.	Discharge of conidia lessens. Some of the first conidia discharged and scattered have reached the water held in the unrolling leaves or in the leaf axils of young susceptible plants, and there have germinated and started infection.
4 to 5 a. m.	Few conidia are discharged or further conidiophores developed but germination and infection by conidia already spread continues.
5 to 6 a. m.	Sun rises and soon dries the plant surfaces, killing the few conidiophores and conidia still developing from infected plants, and also the conidia which have not yet achieved the infection of the new hosts they have reached. Those conidial germ tubes, however, which have already penetrated in to the host tissue are not killed when the plant surface is dried but continue to invade the host within whose tissues they are protected.

The procedure which has just been outlined is beautifully adapted to the limitations of the delicate conidia which when dried are killed immediately. Borne on conidiophores which are submerged in dew, carried in countless numbers through the cool darkness on currents of damp air, dropped into the moisture contained by such susceptible parts as unrolling leaves, and there sending germ tubes into the succulent interior tissue of the host, the conidia, despite their delicacy, are able to accomplish infection in a remarkably efficient manner.

This procedure is also exceedingly successful in accomplishing the rapid spread of infection and distribution of the disease. Plants thus diseased will in turn begin producing conidiophores after about 10 days or, if they are young, even in 3 to 5. As a result, a small center of inoculation can under favorable conditions cause the infection of large areas in a relatively short time.

DURATION OF CONIDIOPHORE PRODUCTION

The development of conidiophores and conidia from the downy mildew mycelium ensconced within the diseased maize plant is not limited to one night, nor does it cease after it has occurred several nights, but

rather may continue to take place every favorable night for a month, six weeks, or even so great a proportion of the plant's existence as two or more months. Because this has not been realized hitherto, the danger of even a single conidium-producing plant to the surrounding fields has never been sufficiently appreciated or emphasized.

The duration of nightly conidium production was studied in a large number of infected plants, including maize, teosinte, sorghum, sugar cane, *Saccharum spontaneum*, and *Miscanthus japonicus*. At first the plants were examined each night, but later the following more convenient method was used. On the badly infected leaves of each plant an area, on which formation of abundant conidiophores occurred nightly, was outlined on both the upper and lower surfaces with waterproof ink. Each afternoon these areas were carefully scraped clean from all remains of previous conidiophore production; and early the following morning the presence or absence of conidiophores and conidia showed whether the fungus had been active during the night. The lengths of the periods of nightly conidiophore production in several typical cases of infected maize are presented in Table I.

A series of such observations extending through the wet, dry, and transitional seasons brought out the following points. The length of time during which nightly production of conidia may take place on maize depends primarily on the time normally required by the host to attain maturity, on the vigor of the individual attacked, and on its age when infected. In the case of maize varieties which mature most rapidly, such as the "dwarf early" sweet and pop corns (60 to 70 days), or the Chinese or Mindanao waxy endosperm maize (70 days), the time over which nightly conidium formation may extend is relatively short, usually not over a month. In maize varieties, which mature more slowly, such as the several native flint types which are grown throughout the Philippines and other tropical places, the time is somewhat longer; while those large hardy, dent varieties and flint-dent crosses which require the longest time (3 to 4 months) to mature support the formation of conidia by the parasite for the longest period, for even more than 2 months. Apparently while the host is actively growing and developing, its internal condition is such as to favor both the growth of the parasite and the abundant production of the conidia; but when the host plant has matured, and its vitality begins to decline, its tissues apparently undergo chemical and physical changes which render them unfavorable for the further development and activity of the mildew.

The vigor of the individual plant infected also affects the duration of conidium production. Weakling plants, which result from planting imported seed of foreign varieties unsuited and unacclimatized to Philippine conditions, succumb to the downy mildew in a comparatively short time; and conidium production by the parasite, although exceedingly abundant for a while, is soon brought to an end by the untimely death of the host. On the other hand, if strong, vigorous hardy plants are attacked they do not succumb but continue to support the persistent parasite even for as long as the lifetime of a healthy plant (Pl. 5, B). In some plants, after many weeks of abundant nocturnal spore production, conidia were still found developing on the middle and still green part of the last or next to last leaf, although all the other leaves and even the tips of these sporulating leaves themselves were drying and brown.

TABLE I.—*Duration of the conidiophore-producing period in various infected maize plants, compared with the time usually required by healthy plants of these varieties to mature*

Plant No.	Date planted.	Date emerged.	First symptoms observed.	First conidiophore production observed.	Last conidiophore production observed.	Duration of conidiophore producing period.	Time required for maturing.	Proportion of conidiophore production period to life of plant.	Number of nights conidiophore production observed during period.	Condition of plant at last conidiophore production.
MANOBO WAXY MAIZE										
						Days.	*Days.*	*Per cent.*		
13/2	Sept. 7, 1918	Sept. 10, 1918	Sept. 30, 1918	Oct. 1, 1918	Nov. 5, 1918	36	70	51.42	18	Stunted, maturing.
11/2	do	do	Oct. 8, 1918	Oct. 9, 1918	Nov. 1, 1918	24	70	34.28	13	Maturing.
1/3	do	do	do	do	Nov. 5, 1918	28	70	40.00	13	Do.
4/2	do	do	Oct. 16, 1918	Oct. 17, 1918	do	20	70	28.57	10	Do.
2/3	do	do	Oct. 17, 1918	Oct. 18, 1918	Nov. 1, 1918	15	70	21.42	7	Do.
EXTRA EARLY ADAMS SWEET										
1/1	Jan. 4, 1919	Jan. 8, 1919	Jan. 22, 1919	Jan. 24, 1919	Feb. 19, 1919	27	80	33.75	Not recorded	Stunted.
4/2	do	do	Feb. 12, 1919	Feb. 13, 1919	Feb. 27, 1919	15	80	18.75	do	Do.
GUAM WHITE FLINT										
1/4/a	Dec. 1, 1918	Dec. 6, 1918	Dec. 16, 1918	Dec. 17, 1918	Feb. 18, 1919	64	90	71.11	Not recorded	6½ feet tall with elongate ear branch.
MANGONON WHITE DENT										
2/2	Jan. 29, 1919	Feb. 2, 1919	Mar. 1, 1919	Mar. 2, 1919	May 10, 1919	70	100	70.00	Not recorded	Drying, mature.
3/1	do	do	Feb. 23, 1919	Feb. 24, 1919	do	76	100	76.00	do	Do.
3/2	do	do	Mar. 2, 1919	Mar. 3, 1919	do	69	100	69.00	do	Do.
3/3	do	do	Feb. 26, 1919	Feb. 27, 1919	April 28, 1919	61	100	61.00	do	Do.
4/2/1	do	do	Feb. 22, 1919	Feb. 23, 1919	do	65	100	65.00	do	Barren, drying, stunted.
4/2/2	do	do	Feb. 27, 1919	Feb. 28, 1919	do	60	100	60.00	do	Do.
4/3	do	do	Feb. 23, 1919	Feb. 24, 1919	do	64	100	64.00	do	Do.
4/4	do	do	Mar. 1, 1919	Mar. 2, 1919	May 10, 1919	70	100	70.00	do	Barren, stunted, slender.
5/3	do	do	Mar. 2, 1919	Mar. 3, 1919	April 28, 1919	57	100	57.00	do	Drying, barren.

KANSAS SUNFLOWER YELLOW DENT										
2/1	Feb. 6, 1919	Feb. 11, 1919	Feb. 24, 1919	Feb. 25, 1919	April 28, 1919	63	110	57.27	Not recorded	Barren, drying.
2/3	do	do	Mar. 22, 1919	Mar. 24, 1919	do	36	110	32.72	do	Do.
3/1	do	do	Mar. 5, 1919	Mar. 6, 1919	do	54	110	49.09	do	Do.
HILDRETH YELLOW DENT										
3/1	Feb. 6, 1919	Feb. 11, 1919	Mar. 1, 1919	Mar. 2, 1919	April 28, 1919	58	120	48.33	Not recorded	7 feet tall, tassel.
3/4/1	do	do	Mar. 6, 1919	Mar. 7, 1919	do	53	120	44.16	do	6 feet tall, tassel.
3/4/2	do	do	Mar. 5, 1919	Mar. 6, 1919	do	54	120	45.00	do	5 feet tall, tassel, and small ear.

The age of the plants at infection is also of importance in relation to the duration of conidium production. Plants which become infected when very young (from the time they emerge until they are a few days old) usually succumb more rapidly to the mildew than those which have had some weeks of healthy growth before they are attacked. This is shown very clearly by the observations comprised in the following summary.

In Chinese Waxy maize, planted Nov. 27, 1918, up Dec. 2, matured Mar. 3, 1919:

Of 34	plants showing Sclerospora between	Dec. 17 and 20,	33	died between Dec. 28 and Jan. 16,	1	survived into February.
Of 50	do	Dec. 21 and 24,	38	do	12	do.
Of 21	do	Dec. 25 and 31,	9	do	12	do.
Of 30	do	Jan. 1 and 5,	3	do	27	do.
Of 3	do	Jan. 6 and 10,	1	do	2	do.
Of 7	do	Jan. 11 and 15,	0	do	7	do.
Of 3	do	Jan. 16 and 20,	0	do	3	do.
Of 1	do	Jan. 21 and 25,	0	do	1	do.

In Laguna Mexican maize, planted Nov. 29, 1918, up Dec. 11, matured Mar. 20, 1919:

Of 34	plants showing Sclerospora between	Dec. 17 and 21,	34	died between Dec. 28 and Jan. 16,	0	survived into February.
Of 11	do	Dec. 22 and 26,	9	do	2	do.
Of 5	do	Dec. 27 and 31,	3	do	2	do.
Of 20	do	Jan. 1 and 5,	10	do	10	do.
Of 10	do	Jan. 6 and 10,	2	do	8	do.
Of 4	do	Jan. 11 and 16,	0	do	4	do.

In Kansas Sunflower Yellow Dent, planted Feb. 6, 1919, up Feb. 11, matured May 30, 1919:

Of 11	plants showing Sclerospora between	Feb. 25 and 28,	10	died between Mar. 25 and Apr. 15,	1	survived into May.
Of 1	do	Mar. 1 and 5,	0	do	1	do.
Of 1	do	Mar. 6 and 25,	0	do	1	do.

In Mangonon White Dent, planted Jan. 29, 1919, up Feb. 2, matured May 10, 1919:

Of 8	plants showing Sclerospora between	Feb. 16 and 20,	8	died between Mar. 1 and Mar. 25,	0	survived into May.
Of 11	do	Feb. 21 and 24,	8	do	3	do.
Of 9	do	Feb. 25 and 28,	7	do	2	do.
Of 4	do	Mar. 1 and 4,	0	do	4	do.
Of 1	do	Mar. 5 and 25,	0	do	1	do.

In Yucatan pop corn, planted Dec. 3, 1918, up Dec. 10, matured Mar. 31, 1919:

Of 16	plants showing Sclerospora between	Dec. 19 and 24,	14	died between Dec. 28 and Jan. 10,	2	survived into February.
Of 23	do	Dec. 25 and 31,	19	do	4	do.
Of 3	do	Jan. 1 and 4,	0	do	3	do.

Moreover, such early-infected plants are usually much more liable to the destructive attacks of such secondary parasites as Pythium, Fusarium, Helminthosporium, and bacteria.

Occasionally the period of conidium production is prolonged by the abnormal growth of the diseased plant. In response to some stimulus exerted by the parasite, various parts of the host, especially the shank, husks, ear, and tassel, may develop abnormally, the activity of the mycelium keeping pace with this unduly extended growth of the plant body. In one striking case of this sort, a plant of Guam maize (Pl. 5, B) which showed symptoms of infection after vigorous growth had well begun, supported nightly conidium production by the downy mildew from the time it was 17 days old during its subsequent development. After about 5 weeks, when a normal tassel had been produced, the very young ear began to develop; and, abnormally stimulated by the downy mildew mycelium which had invaded the bud as it formed, the shank grew excessively in length, forming a branch which was clothed with extensive leafy husks, almost equaled the main axis in length, and bore at its tip a small sterile ear. The remarkable growth of this abnormal ear branch occupied about 30 days. The branch was still green and vigorous long after the healthy plants nearby were dry, and about 14 days after the upper part of the main axis had begun to wither. During all this time nightly conidium formation by the parasite was taking place from all parts of the excessively developed leafy husks of the abnormal branch, thus prolonging the period of conidium production by the infected plant over a total of more than nine weeks. Finally, about two weeks after conidium formation was observed for the last time, the vitality of the branch declined and it gradually withered.

The behavior of the downy mildew on its other hosts presents an interesting comparison to that which is characteristic for maize. In the few sorghum plants that became infected, the period of conidium production was necessarily brief, as death occurred a few weeks after infection. This was true also in the only cases of conidially infected sugar cane which were encountered (*25*). Moreover, on both these hosts the nocturnal conidium production during this brief period was far less extensive and abundant than in maize. In teosinte, on the contrary, the duration of conidiophore production is prolonged beyond that of maize, because of the constant renewal of young tissue suitable for spore production. As new shoots are sent out from the base of the infected plant (Pl. 6, A, C), or as new branches arise, the fungus mycelium of the main axis grows out into the freshly formed tissue, keeping pace in its development with the renewed growth of the plant, on which it produces additional conidiophores. In this way conidium production, although not so abundant on any one night as in maize of equal age, is prolonged and continues constantly and persistently for several months. This was also true in teosinte-maize hybrids, which showed extensive growth of numerous suckers and branches. In *Miscanthus japonicus* the period of conidium production was similarly prolonged. It is in *Saccharum spontaneum*, however, that this prolongation is greatest. Infected plants kept under observation were still producing conidia on many newly formed shoots even after an uninterrupted period of over eight months (*25*). Moreover, conidium production was renewed on new shoots arising from the well-established rootstocks of infected plants even

after the plants either when young or when quite passé were cut back to the ground surface. Thus conidium formation by the parasite on *Saccharum spontaneum* can be as persistently perennial as the grass itself.

QUANTITY OF CONIDIUM PRODUCTION

The number of conidia produced from a single maize plant infected with downy mildew even during one night is exceedingly great, while the total conidium production from such a plant throughout the entire season is appallingly vast. It is necessary to consider this matter further in order to appreciate the infectious menace to a maize-growing district which is embodied in even a single diseased plant. The amount of conidium production from any one plant during any one night depends on three factors: First, the extent on the host of the discolored area which is most heavily invaded with mycelium and especially active in conidiophore production; second, the number of stomata in both surfaces of this area; and third, the proper environmental conditions to induce conidium formation.

The number of conidia borne by a plant is directly proportional to the extent of the yellowed, whitened, or otherwise symptomatically discolored part of its surface. Although the hyphae of the parasite are to some extent found in and may give rise to conidiophores on the apparently unaffected parts of the host, it is from the discolored areas which have become etiolated through the destructive invasion of abundant mycelium of the fungus that the great mass of conidiophores emerges. The development and progressive increase in extent of this abundantly conidiophore-bearing surface has been studied both in inoculated plants grown under controlled conditions and in naturally infected plants in the field. The procedure is as follows: After the young plant is infected, there ensues a latent period of from a few days to more than two weeks, its length being directly proportional to the age of the plant when inoculated. The mycelium spreads throughout the plant tissues and concentrates especially in those areas of the leaves and leaf sheaths which begin to show symptomatic etiolation. The production of conidiophores on the whitened areas then commences. At first the conidiophores appear scatteringly (Pl. 1, A; 4, A, B), but production gradually increases during subsequent nights until the affected areas are covered with a dense down of conidiophores (Pl. 4, C, D). In proportion as new leaves unroll from the bud, and as the leaves, which were already partly developed when conidium production began, continue to elongate by intercalary growth, the conidiophore-bearing surface is constantly augmented (Pl. 1, C). Finally, when all the leaves are fully developed, the production of conidiophores reaches its maximum. Even then, in badly infected cases, this production may be still further increased by the formation of conidia from the tassel, from the husks, which are often abnormally developed (Pl. 5, B), and from the peculiar bractlike structures which frequently form in abnormal tassels (Pl. 5, A) or around abortive ears. Thus the conidiophore-bearing surface, which may be established when the infected plant is only about a week old, and which at first may be only a few square centimeters in extent (Pl. 2, A; 3, A), gradually increases in area (Pl. 2, B; 3, B) until it attains its maximum of some two or three thousand square centimeters; and then, decreasing in extent as successive

leaves of the maturing host wither, it finally ceases to support conidium production after months of activity. The extent of the discolored conidium-bearing areas on plants of various ages has been determined for the different maize types most common in the Philippines. The least area is found in the case of such small, rapidly maturing varieties as Manobo and Davao Waxy Maize and the dwarf native flints. The maximum area occurs in such large, slowly maturing types as introduced American dent varieties or those strains, such as "Moro" White, developed from dent-flint crosses. Between these two extremes lie the intermediate areas which are typical of the medium-sized, moderately rapidly maturing yellow and white flint types that are so commonly grown throughout the islands.

In order to gain an idea of the extent of the conidium-producing areas of diseased plants under the usual field conditions, measurements were made of characteristically infected specimens both of the common native-grown maize and of the introduced types under cultivation at the college. Some of these measurements are shown in Table II. In obtaining these data the following method was used: Leaves bearing conidiophore-producing areas were carefully removed from infected plants and pinned down to large sheets of paper upon which the outline of the whitened areas was carefully traced through the tissue of the leaf with a sharp stylus. The space inclosed within these tracings was then computed by means of a polar planimeter. Only portions which were found by actual observations to be producing conidiophores were included. In the specimens tabulated, therefore, the measurements give a fair idea of the amount of surface on which conidiophores were borne. The extent and general configuration of this surface on the successive leaves of two of the plants included in Table II are shown in Figure 1.

To the second factor, the number of stomata in the conidium-bearing surface, the quantity of conidia produced is also directly proportional. In no case were conidiophores developed except from the stomata, and in the manner already described. The stomatal frequencies recorded by Eckerson (*8*), Duggar (7), Kiesselbach (*13*), and others show a wide range of variation not only between different individuals or varieties under similar or diverse environmental conditions but also between different parts of the same plant. In the present instance, therefore, no extensive counts were made, but enough material was examined to determine that in the principal types of infected maize studied in the Philippines the stomatal frequencies fall easily within this range. In such varieties as Manobo Waxy, the number agreed closely with Kiesselbach's counts for Chinese Waxy; while in the large flint-dent crosses, such as Moro White, the number closely approximated his counts for various dent types. In general, it was found that his averages of 77 and 93 per square mm. of upper and lower surface were sufficiently conservative to serve as fair mean values for calculation in the Philippine maize varieties studied.

TABLE II.—*Conidiophore-bearing areas on representative maize plants of various ages infected by downy mildew*

NATIVE YELLOW FLINT

No.	Approximate age.	Number of leaves.	Approximate height.	Whole area. Blades.	Whole area. Sheaths.	Whole area. Total.	Conidiophore-bearing area. Blades.	Conidiophore-bearing area. Sheaths.	Conidiophore-bearing area. Total.	Percentage conidiophore-bearing.	Conidium production.
	Weeks.		*Cm.*	*Sq. cm.*	*Sq. cm.*	*Sq. cm.*	*Sq. cm.*	*Sq. cm.*	*Sq. cm.*		
1	2 to 3	6, seventh unfolding	67.0	786.00	144.50	930.50	372.70	3.74	376.44	40.45	Very heavy.
2	4	7, eighth unfolding	84.0	1,130.64	258.84	1,389.48	551.40	42.00	593.40	42.70	Do.
3	4	9, tenth unfolding	93.0	1,402.15	369.66	1,771.81	589.04	31.42	620.46	35.01	Medium.
4	10	8, and tassel	101.0	1,409.07	426.50	1,835.57	723.30	71.56	794.86	43.30	Heavy (stunted plant).
5	4 to 5	8, ninth unfolding	91.0	1,819.02	349.15	2,168.17	351.70	39.77	391.47	18.05	Very heavy.
6	7	9, tenth unfolding	115.0	2,052.50	392.68	2,445.18	835.00	12.54	847.54	34.66	Do.
7	10	9, and tassel	122.0	2,154.33	539.71	2,694.04	1,145.45	98.93	1,244.38	46.19	Do.
8	7 to 8	11	120.5	3,166.47	520.66	3,687.13	851.60	22.08	873.68	23.69	Medium.
9	8 to 9	12, and tassel	126.0	4,112.09	790.35	4,902.44	1,146.05	161.16	1,607.21	32.78	Heavy.
10	10	....do	192.0	5,127.90	1,371.70	6,499.60	2,629.20	571.08	3,200.28	49.23	Very heavy (full-sized plant).

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No.	Approximate age.	Number of leaves.	Approximate height.	Whole area. Blades.	Whole area. Sheaths.	Whole area. Total.	Conidiophore-bearing area. Blades.	Conidiophore-bearing area. Sheaths.	Conidiophore-bearing area. Total.	Percentage conidiophore-bearing.	Conidium production.
11	10 to 11	9, and tassel	126.0	1,502.78	429.92	1,932.70	1,346.55	96.99	1,443.54	74.69	Medium.
12	6 to 7	9	115.0	2,268.68	352.15	2,620.83	1,049.30	12.45	1,061.75	40.51	Very heavy.
13	7 to 8	9, and tassel	136.0	2,193.61	323.97	2,517.58	1,001.25	173.44	1,174.69	46.65	Heavy.
14	7	9, tenth unfolding	135.0	3,228.39	530.30	3,758.69	1,511.40	30.60	1,542.00	41.02	Very heavy.
15	8	11	138.0	3,229.19	855.22	4,084.40	1,934.48	470.71	2,405.19	58.88	Do.
16	9 to 10	9, and tassel	154.0	3,656.79	926.32	4,583.11	1,285.80	114.84	1,400.64	30.56	Medium.

The third factor, environmental conditions favorable for conidium formation, bears on the quantity of conidia produced as follows: Under the usual field conditions, when the infected plants have begun to show the characteristically discolored areas, and these have begun to bear conidia, the number borne is at first comparatively small, as the conidiophores are few and scattered. As time goes on, however, and conidiophore production is induced night after night successively, production rapidly increases until on the area already established it attains its maximum density. This maximum density under such favorable environmental circumstances may occur even before this area has reached its maximum size. On the contrary, if such a plant has been exposed to conditions preventing conidiophore production, the area, even though long established, will not produce large numbers of conidiophores until favorable conditions have prevailed for so long a time that conidium production has been enabled to build itself up to the maximum.

It is apparent, therefore, that a plant to produce a maximum quantity of conidiophores must have: First, a large conidiophore-bearing surface; second, a large number of stomata per unit of this surface; and third, continued favorable conditions which permit conidiophore production to increase night after night to its maximum. These requirements are fulfilled in such typically Philippine varieties as Moro White, Native Yellow, and Cebu White, or in the even larger introduced dent and flint types. After studying conidiophore production on such varieties at night, and finding plant after plant with the greater part of its leaf surface white with a felt of densely grouped conidiophores (Pl. 4, C, D), and after seeing the thousands of spores which fill even a minute droplet scraped from the surface of such a leaf, one is impressed by the vast production of conidia which goes on. To get a more definite conception of this vast production the writer made approximate estimates of the number of conidia formed on typical plants. These estimates were calculated as follows: The total surface bearing conidiophores was multiplied by the number of bearing stomata in a unit of surface, and this result by the average number of conidiophores from each stoma, and this in turn by the average number of conidia per conidiophore. For the conidiophore-bearing surfaces the measurements recorded in Table II were used. For the number of stomata the averages of Kiesselbach were employed, but as microscopic examination showed that usually only about one-half the stomata actually bore conidiophores the averages, 77 above and 93 below per square millimeter of surface, were reduced accordingly. The number of conidiophores bearing conidia at each stoma when examined at different times during the night was found to vary from one to four, with two as a conservative average. From the number of club-shaped initials in various stages of growth at each stoma the number of conidiophores successively developed per stoma during the nightly cycle was approximately estimated as six or three successive "crops" of two each. The number of conidia on the conidiophores, although very variable, most commonly ranges from 32 to 48; 40, therefore, was selected as a fair average. The results of the calculations are shown in Table III.

These figures may serve to give a clearer idea of the amount of conidium production on a diseased plant than can be obtained merely from description. They are, of course, only approximate; but they are extremely conservative, and purposely have been made to underestimate rather than overestimate the number. Obviously a single diseased plant, even a

small one and one of relatively low spore production, is able to develop dangerously large quantities of conidia each night; while a large plant with maximum spore formation in its nocturnal cycle liberates a startingly vast number of conidia. From extensive, badly infested maize fields in which 50, 70, or even more than 80 per cent of the plants are heavily conidiophore-bearing, an incredibly large number of conidia is produced. Moreover, it should not be forgotten that this production continues night after night for months, thus achieving a total output of conidia that is almost unbelievable. With the magnitude of this spore production in mind it is easy to understand the rapid spread of the downy mildew under Philippine conditions from even small isolated centers of infection, and to appreciate the menace to the whole maize-growing countryside which one infected field or even one single badly diseased plant may represent.

TABLE III.—*Calculation of approximate number of conidia liberated from the conidiophore-bearing surfaces of representative maize plants during one night*

NATIVE YELLOW

Plant No.	Conidiophore-bearing surface.			Average number of stomata per square centimeter.	Average number of maturing conidiophores per stoma at one time.	Average number of conidia per conidiophore.	Number of conidia produced on plant at one time.	Successive "crops" of conidiophores produced during night (average).	Total number of conidia produced on plant during night.
	Leaf surface (2Xarea).	Sheath area.	Total.						
	Sq. cm.	*Sq. cm.*	*Sq. cm.*						
5	703.40	39.77	743.17	4,250	2	40	252,677,800	3	758,033,400
6	1,670.00	12.54	1,682.54	4,250	2	40	572,063,600	3	1,716,190,800
7	2,290.90	98.93	2,389.83	4,250	2	40	812,542,200	3	2,438,126,600
9	2,292.10	161.16	2,453.26	4,250	2	40	834,108,400	3	2,502,325,200
10	5,258.40	571.08	5,829.48	4,250	2	40	1,982,023,200	3	5,946,069,600

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12	2,098.60	12.45	2,111.05	4,250	2	40	717,757,000	3	2,153,271,000
14	3,022.80	30.60	3,053.40	4,250	2	40	1,038,156,000	3	3,114,468,000
15	3,868.96	470.71	4,339.67	4,250	2	40	1,475,487,800	3	4,426,463,400

DISPERSAL OF CONIDIA

When the conidia are being produced their distribution, and the resulting spread of the downy mildew, is accomplished primarily by wind, to a less extent by splashing or wind-blown rain, and to a still less extent by surface water. Obviously, distribution of effective, germinable conidia can take place only at night or possibly, under unusually favorable conditions, until a little after dawn, because the conidia can not survive drying. Obviously, also, any conditions restricting or favoring conidium production in like manner directly affect their dispersal.

DISPERSAL BY WIND

The two Philippine Sclerosporas of maize are admirably adapted to distribution by wind as their conidia are produced in vast numbers and are sufficiently small so that they may be readily carried in the air. The great number of conidia produced has already been considered. The size of the individual conidium, although far larger than that which

characterizes the breeze-borne basidiospores of the Hymenomycetes and slightly larger than that of the wind-carried oidia of the oak mildew, is relatively small, being far less than that of the air-distributed spores of Lycopodium, or of the pollen of anemophilous conifers and grasses. From its dimensions the volume of an average Sclerospora conidium may be calculated roughly as follows: If the spore is regarded as consisting of a cylindrical main portion with hemispherical ends, as is diagrammatically shown in figure 2, the total volume in cubic μ will equal the volume of this cylinder plus that of the two hemispheres. In *Sclerospora philippinensis*, the most commonly encountered length is 33 to 35 μ (34 μ), the most frequent diameter 17 to 19 μ (18 μ); while the greatest length is 52 μ, and the greatest diameter 24 μ. On the basis of these measurements we may calculate the most commonly encountered volume as 7,125.15 cu. μ (0.000,000,007,125 cc.), while the greatest volume is similarly found to be 19,905.18 cu. μ (0.000,000,019,905 cc.). In like manner, in *S. spontanea*, the most commonly encountered length is 42 μ and the diameter is 16 μ, while the greatest length is 64 μ and the greatest diameter 20 μ, giving a most common volume of 7,372.29 cu. μ (0.000,000,-007,372 cc.), and a greatest volume of 18,011.84 cu. μ (0.000,000,018,011 cc.).

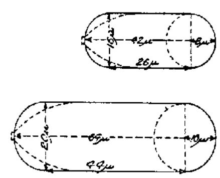

FIG. 2.—Diagrams representing *Sclerospora spontanea* conidia of the most common and of the largest size schematized to facilitate calculation of approximate volumes.

The specific gravity of the conidia was not determined. It was observed, however, that the spores, although they occasionally float, usually sink very gradually in a water mount. It may probably be assumed without serious error that their specific gravity is little if any greater than that of the heaviest Hymenomycete spores (*Coprinus plicatilis*, 1.43) studied by Buller (*2*). Their weight, therefore, is relatively very small.

The rate of fall of the Sclerospora conidia was not determined. The fall of these conidia, because of their larger size, would undoubtedly be faster than the velocity of 0.429 cm. a second, found by Buller for Coprinus; but it would not, in all probability, exceed markedly the 1.76 cm. a second determined by Zeleny and McKeehan (*26*) for Lycopodium spores which have a specific gravity of 1.175 and a diameter of 31.6 μ. Furthermore, because Buller found that the elongate spores of *Polyporus squamosus* when falling soon assumed a position with the long axis horizontal and thus were retarded by the increased air resistance, it is probable that the similarly shaped Sclerospora conidia, even though larger, behave in like manner. In any case it seems justifiable to assume that the rate of fall of the conidia is sufficiently slow to facilitate their being carried by breezes of even slight strength. Judging from the foregoing, conidia, even in absolutely still air, when shed from the upper leaves of maize plants at a height of 1.76 meters would require nearly three minutes (176 seconds) to reach the ground, and that from a height of 3.52 meters, which is not infrequently attained by conidiophore-bearing tassels and topmost leaves of large maize varieties, the conidia would take but little less than six minutes to complete their fall.

Moreover, Schmidt (*21*) shows that widespread distribution of a large proportion of liberated spores is accomplished by winds of low velocity. He calculates that Lycopodium spores which have a rate of fall of 1.76 cm. a second, when liberated in a wind blowing 36 kilometers (22.5 miles)

an hour possess an average limit of distribution of 330 kilometers (206.25 miles). Similarly even in the case of *Pinus sylvestris* pollen, which has as great a diameter as 48 μ, and as rapid a fall as 5.3 cm. a second, he computes that as many as four-tenths of the number originally shed would be carried 4 kilometers by a wind of 36 kilometers an hour; while two-tenths would reach 13 kilometers; one-tenth would reach 20 kilometers; and one-hundredth would reach 36 kilometers. The work of Hesselman (*12*) shows that these calculations represent the conditions attained in nature. He found that conifer pollen grains 30 to 60 μ in diameter were deposited in relatively vast numbers in dishes on light ships 30 kilometers (18.6 miles) and 55 kilometers (34 miles) from land; while pollen of grasses also, in spite of being liberated in far less abundance, nearer the earth, and unequipped with appendages for buoyancy, was caught in considerable quantities.

It seems justifiable to conclude from the foregoing evidence that the conidia of Sclerospora also are easily and effectively distributed by air currents. The writer's observations also bear this out. Small, infected maize plants, known to be producing abundantly, were covered at dusk with large cans or bell jars the edges of which were pressed into the earth and sealed with clay. The nocturnal fall of innumerable conidia then took place undisturbed except by such slight convection currents as were operative within the can. As a result Petri dishes placed on the ground under the plant, or, even the ground itself, showed a heavy deposit of spores which in some cases was sufficient to whiten the earth conspicuously. When, however, the plants were left exposed to the wind, no such abundant spore deposit was found under them even after apparently calm nights; and after nights with gusty winds almost all the conidia were carried away, few, if any, being caught in the exposed Petri dishes under the plant. Further observations and experiments concerning the distribution of conidia by winds of different velocities will be given in the following sections.

The dispersal of the conidia by wind is of three main types; first, by very slight local air currents; second, by more general breezes of greater strength; and third, by violent and extensive typhoons.

BY SLIGHT AIR CURRENTS

The slight air currents, although very gentle and necessarily restricted in extent, are very important, because of their invariable occurrence, in distributing the conidia locally. Even during nights when the air is apparently absolutely still, careful scrutiny will show that here and there a corn leaf is moving slightly in air currents otherwise imperceptible. These nightly driftings of the air are necessarily confined to restricted areas and are influenced in direction and extent by very local conditions. At the College of Agriculture of the Philippines where, for the most part, the dispersal of conidia was studied, the influence of Mount Makiling was the most noticeable factor. By this heavily forested mountain, which rises to a height of over 3,300 feet, the air is cooled at night and flows down the slopes to the plain. The presence and direction of this air flow were determined under varying night conditions by repeatedly observing the course and velocity of buoyant seeds, such as those of Ceiba or Saccharum, which were thrown into the air and followed by the beam of an electric torch. Field observa-

tions showed that the spread of the downy mildew in the corn plantings closely followed this air flow in direction and extent. Plots containing recently germinated and extremely susceptible maize seedlings developed few if any cases of the downy mildew when located toward the mountain to windward of actively infective plants. Similar plots, however, situated away from the mountain to the leeward of the same actively infective plants and in the direct path of the stream of conidia borne on the nightly air currents from them developed a very high percentage of downy mildew cases.

In larger fields it was possible also to demonstrate that the spread of the downy mildew closely followed the direction and extent of the nightly air currents. When, after carefully removing any actively infective plants so situated as to be dangerous to such a field, a single conidium-producing plant was transplanted to the center of it, that portion of the field located to windward of the diseased plant remained free from disease except in the very immediate vicinity of the plant itself. In the leeward part of the field, however, as the young corn developed, there subsequently appeared numerous cases of downy mildew. These were especially frequent along the fan-shaped path of the conidia which were carried by the nightly air currents from the centrally placed diseased plant and were increasingly numerous the nearer one approached the source of infection. Although, as has been mentioned, the general trend of these nightly air currents was down from the mountain, it was found by experimenting that their extent and direction were greatly influenced by local conditions. Such topographical features as wooded ridges, hills, deep gullies or brook beds, and small bodies of water had a marked effect; while buildings, thick woods, and dense growths of tall cogonal grasses often served also to obstruct or deflect the air currents. Even trees, bamboo or banana clumps, and small plots of sugar cane, sorghum, or cassava were found to disturb the nightly air currents by causing little eddies and local circulations of air.

Petri plates containing a thin film of clear 1 per cent agar were employed to gain a more exact idea of the direction and extent of conidium dispersal by these nightly air currents. On an apparently still night such plates were exposed during the period of maximum conidium formation at various distances around a single diseased plant which was producing abundant conidia. All other actively infective plants in the vicinity having been removed, careful record was made both of the position of the plates and of the direction of the prevailing air currents in relation to the diseased plant. At dawn the plates were carefully examined under the microscope and the presence and number of conidia noted. Very few spores were found in plates situated to windward of the infective plant, and only in those which had been very near it (within 3 or 4 feet). To the leeward of the infective plant, however, and directly in the path of spore distribution especially, large numbers of conidia were caught. These were most numerous in plates near the plant and proportionately less abundant in those at greater distances. Plates within 8 or 10 feet were liberally showered; those 20 and 30 feet distant showed many conidia; while those situated 50, 60, and in one case, even 80 feet away still caught a few. As the Petri dishes were of small size (10 cm. diameter) it was to be expected that at greater distances the conidia would be so scattered as to reduce to the minimum the chances of their being captured. It should be noted that the conidia whether caught at 8 or 80 feet germinated vigorously on the moist agar surface.

BY BREEZES OF GREATER STRENGTH

Dispersal of conidia is accomplished also by breezes and night winds of moderate strength. By these the conidia are carried more rapidly and to a far greater distance than by the gentle, almost imperceptible air currents just considered. At the College of Agriculture at Los Banos stronger winds and breezes play a lesser part in the total conidium distribution than does the nightly air drift, because they are relatively infrequent, and often occur when storms are in the vicinity and when the sky is cloudy so that no conidia are produced. Occasionally, however, gusts of brief duration but of considerable strength were noticed during nights when abundant spore production was taking place. These were most noticeable just before dawn. Spore dispersal is most effectively accomplished when the breeze comes in such occasional intermittent puffs, as when constant it tends to dry the dew from leaf surfaces and thus kill the conidiophores that are being produced.

In certain localities, where conditions are more favorable, stronger breezes occur with great regularity when conidia are being formed. In regions near the coast the on-shore breeze dies down after sunset; and as the earth cools, an off-shore breeze springs up, starting at a considerable distance inland, and blowing toward the sea. This was particularly noticed in the vicinity of Lingayen Gulf, Pangasinan Province, where a distinct off-shore breeze was found to prevail at night, sweeping from far inland over the maize fields toward the shore. In extensive flat plains also, noticeable breezes arise at night and sweep over considerable areas even on nights when sufficient dew formation takes place to insure the abundant formation of conidia. This was found to be the case in the broad plain which runs from Batangas and Balayan Bays northwest through Batangas and Laguna Provinces to the Lake of Bay. Throughout this plain where corn occupies about 31,000 acres, a large proportion of the cultivated land, and where the downy mildew is exceedingly destructive, such breezes were found to be a very important factor in the distribution of the downy mildew. Observations were made at Taal in the midregion of this plain, at Calamba, near the northern limit, and at Batangas on the southern coast; and in each place marked breezes were noted on nights when conidium production was occurring. At Batangas the off-shore breeze to the bay also influenced the spore dispersal, while at Taal and Calamba the effect of the presence of the lakes was noticeable.

BY VIOLENT GALES

Dispersal of conidia is accomplished by night winds of high velocity and great strength, such as those occurring during severe tropical storms. At first it seemed doubtful to the writer if conidium production could take place under such conditions even though leaves were moist. Accordingly, plants known to be producing abundant conidia on normal dewy nights were observed at night during storms of varyin degrees of violence. These observations showed that, on the whole, conditions which prevailed during such storms were unfavorable to conidium formation. Frequently the torrents of driving rain and the terrific gusts of wind lashed the leaves about so violently, or even beat down the whole plant so completely into the mud, that the delicate conidiophores were prevented from developing. During less violent storms, the rain, although sufficiently gentle in itself to permit conidium formation, was

frequently intermittent and alternated with gusts of wind which blew the water from the leaves and dried the developing conidiophores sufficiently to kill them. None the less conidium production could and did take place during these tropical storms despite their unfavorable violence. In all but the most destructive typhoons at least a few plants in each plot were sufficiently sheltered from mechanical injury so that their leaf sheaths, the under sides of midribs at the base of the leaves, or other protected parts, permitted the production of considerable numbers of conidia. In some cases conidium formation actually took place on the inner surface of leaf sheaths (Pl. 4, E), husks, or bracts which were so situated that they shed direct beating rain. Moreover, the storms frequently began with a cold rain which fell steadily for from one to about three days, and the wind, when it arrived, came first in intermittent gusts before increasing to its full strength. Under these conditions conidia were produced in abundance on large numbers of plants and for several hours were given ample opportunity to be distributed by the wind before its violence was sufficient to injure the developing conidiophores.

As a concrete example of conidium production under unfavorable storm conditions observations made in the typhoon of December 25 and 26, 1918, are of interest. This storm was an unseasonable one, occurring at the beginning of the dry season, and preceded as well as followed by calm, dry weather. It was also a violent one. During December 25 and 26 the rainfall was 59 mm. and the wind, by anemometer, blew 262.8 miles, while during the rest of the month the rainfall averaged 1.17 mm. and the wind 62.2 miles, a day. Cloudy, sultry, threatening weather immediately preceded the storm which began in the afternoon of December 25 with occasional gusts of wind and squalls of rain. The temperature, which during the hottest part of the day, had reached 82° F., decreased rapidly, dropping after sunset to 70°. The plants under observation were well covered with moisture despite the increasing violence of the storm. By 11 o'clock that night the rain fell heavily and almost continually, while the wind was violent but intermittent. From 1 to 2.30 a.m., December 26, the storm raged at its height with rain and wind of such terrific force that examination of the plants was impracticable. After this, however, a gradual decrease was apparent, and by 4 a.m. the rain and wind had lessened, although violent squalls occurred from time to time. The maize plots were then examined. Many plants, both healthy and diseased, had been blown flat and beaten into the wet earth. However, several heavily infected plants which had been under observation showed conidiophore production in abundance on the better protected parts of their leaves and sheaths. Microscopic examination showed many conidiophores which had already discharged their conidia, hence production had probably been going on for about two hours and even during the worst of the storm. Formation of conidia continued until about 6 a.m. when it was checked as the plants began to dry. During this time the wind continued to blow in gusts favorable for spore dispersal. By afternoon the storm had disappeared.

It was, of course, impracticable to determine the precise amount or extent of dispersal of conidia during storms by any such direct means as Petri dishes. Observations in the field, however, showed repeatedly that in regions where the disease had been spreading slowly on the slight nightly air currents, a sudden scattering of the disease appeared, after the normal incubation period following the storm, for several miles along the track of the typhoon.

Wind, in so far as the writer was able to determine, is the most important factor in scattering the conidia of the Philippine maize mildews. In the other cereal downy mildews of the Orient various methods of conidium dispersal have been emphasized by previous investigators. Unfortunately, Butler's (*3*) discussion of the downy mildew of maize in India does not consider this point. With regard to the Sclerospora of corn and sugar cane in Formosa, Miyake (*15*) states that he obtained no direct experimental data on the methods of spread of the conidia in the field because he could not collect spores with germinating power. From field observations, however, he concludes that the spores are distributed by wind, by rain, and by irrigation water; but he regards the distribution of diseased cuttings of cane as the most dangerous and effective method of spread. In the case of the Javan maize mildew, Rutgers (*20*) disregards the matter of conidium dispersal and considers it doubtful if infection by conidia occurs normally in the field in spite of the fact that Raciborski (*19*) had secured infection with conidia and had concluded that they undoubtedly were spread by the wind. Palm (*16*), however, rightly infers from field observations that the disease spreads by the wind dispersal of conidia, and he fully appreciates the importance of this method. It should be noted, however, that his experimental evidence (the finding of conidia in Petri dishes exposed in the path of the wind 10 to 50 meters from a 60 per cent infected field) is valueless. As these plates were exposed during the day and "Het weer was zonnig en droog," the conidia caught were undoubtedly the dried remainder from a previous night's crop, quite dead, and quite incapable of germination or infection. By his experiments "the presence of the conidia in the air was shown" as he says (in translation), but as these conidia were incapable of germination their presence in the air gave no proof of their ability, wind-borne through they were, to spread infection. It would be analogous to reason that certain species of grasses are distributed by ocean currents because the seed, although killed by immersion in salt water, are carried long distances in the sea.

SPLASH DISPERSAL

Dispersal of the conidia is also accomplished by splashing. On dewy nights this is constantly taking place as the large drops of accumulating dew drip from the upper leaves, strike the conidiophore-bearing surfaces of the leaves below and splash off, carrying innumerable conidia to neighboring plants. Droplets thus splashed off, when caught on sheets of glass and examined with the microscope, were found to contain large numbers of conidia which germinated vigorously in dew or on agar. Although the spreading of infection undoubtedly occurs in this direct way, it is of comparatively limited extent and involves only plants in the immediate vicinity. During rains, however, provided conidium production is taking place, the conidia are splashed far greater distances; and, when the splashed-off droplets are carried by the wind, whether by the gentle air currents of still dewy nights or by the gales of violent tropical storms, the distance of dispersal is greatly and correspondingly increased.

The distance over which such droplets are spread either by simple splashing after falling from various heights and upon different kinds of surfaces or by spattering into and being carried away by air currents of various strengths has been studied very thoroughly by Faulwetter (*10*).

From his investigations it seems safe to assume that in the downy mildews of maize splash distribution would be accomplished as follows: Drops of dew dripping upon moist spore-bearing leaf surface only 12 inches below would scatter droplets full of conidia over an area 20 to 32 inches in diameter. Raindrops falling from a height of only 16 feet upon such leaf surfaces 4 feet above ground would spatter droplets containing conidia for a distance of 3 feet 2 inches if the leaf was level or as much as 5½ feet if the leaf surface inclined 30°. Droplets spattered thus from a leaf 4 feet above the ground would be carried as much as 18 feet by a wind blowing 10 miles an hour and proportionately farther by winds of greater velocity. Also conidium-containing droplets would be relayed on from plant to plant by splashing and by wind throughout the duration of a storm. Faulwetter (*9*) demonstrated that spattering and wind-blown rain were important factors in the dissemination of the angular leafspot of cotton. The writer, from a study of the maize downy mildews of the Philippines, is convinced that these factors are operative in their distribution also.

The two methods thus far considered—namely, wind distribution and splash distribution acting either separately or together—are the main instruments in achieving dispersal of conidia of the maize downy mildews under Philippine conditions.

DISPERSAL BY AGENTS OF MINOR IMPORTANCE

Surface water apparently accomplishes conidium dispersal to some extent by washing the conidia which fall in it into contact with young seedlings which are temporarily submerged. Although this was not shown to take place by direct experiment, nevertheless there is considerable evidence of infection occurring in this manner. When glass dishes containing brook water and rain water were exposed at nights under plants producing large numbers of conidiophores, great quantities of conidia were caught. Some of these floated while others sank, but all germinated vigorously. When these dishes were exposed to the sun the conidia were soon killed, probably by the rapid increase in the temperature of the water. When the dishes were protected from the sun, however, the conidia retained their vitality and continued to germinate and grow until some hours after dawn. Young, susceptible plants, inoculated with a concentrated suspension of living conidia from these dishes, soon developed the disease. Moreover, in the field it was frequently noticed that during the rainy season low-lying plots often became submerged under a few inches of standing surface water at a time when the maize had just germinated and the young seedlings were in their most susceptible stage. Extremely heavy infection generally resulted in these inundated areas, the remainder of the field being much less affected. This was also observed by Baker (*1*) who wrote:

> In one interesting case, a part of a field had been subjected to wash during a heavy storm just after planting. On this area every plant was taken by the mildew, while in the remainder of the field not a half dozen died.

Insects also contribute somewhat in conidium dispersal. While diseased corn plants were being examined at night, insects were often seen crawling about on the conidium-bearing leaves and passing indiscriminately from plant to plant. In spite of the fact that many insects are not active at night and that even the nocturnal ones are kept away

to some extent by the moisture on the dew-covered plants, none the less a considerable number were collected on maize during conidium production. These included such diverse forms as several types of leaf-eating larvae, many kinds of ants, and a variety of grasshoppers, leafhoppers, Phryganids, Hemiptera, and Coleoptera. On examination it was found that the appendages and bodies of these insects often had adhering to them large numbers of conidia which germinated readily when placed in dew or on agar. Moreover, in the leaf-eating larvae such as *Plosia* sp., the intestine and even the excrement showed conidia, some of which proved capable of germination when tested. Since the carrying of conidia by insects is apparently of very little importance in comparison to other methods of distribution, no attempt was made to secure further experimental data on frequency of inoculation or distance of spread. It seems clear, however, that occasional infection may occur in this way.

Moist soil with conidia adhering probably serves infrequently as a contributory means toward securing distribution of the downy mildew by other agencies. The damp earth under the low-hanging leaves of small, heavily infected plants occasionally, after still nights, was seen to be whitened with innumerable conidia. Examination showed that these retained their vitality and germinability on the soil until dried by the early sun, while such conidia as were lodged in protecting crannies lived even longer. Moreover, the infection of germinating maize seeds that had not yet emerged from sterile sand was easily accomplished by using bits of this conidium-laden soil as inoculum. The seeds were uncovered, bits of infective soil were placed on their plumules, the sterile sand was replaced, and the pots were protected from further infection. The resulting seedlings were heavily diseased. Undoubtedly seedlings when emerging or just at the surface of the soil are occasionally infected in a somewhat similar fashion in the field. It is probable, also, that such conidium-laden soil is at times splashed about and causes the infection of adjacent maize plants quite as the sporangia of Pythiacystis were found by Smith (23) to reach and inoculate low-hanging lemons in bits of spattered earth. Furthermore, it is possible that infective soil could be carried to susceptible young plants by animals, or implements or men. In practice, however, such obstacles as, first, the necessity that the infective soil should be transported before or shortly after dawn, and, second, the necessity that it should reach the susceptible protected parts of the host, would probably prevent this method of distribution and infection from operating in any save rare instances.

DISSEMINATION OF THE PHILIPPINE SCLEROSPORAS

The foregoing discussion of conidium production and dispersal logically leads us to consider the broader subject of the dissemination of the Philippine Sclerosporas. The dissemination of such fungi may be conveniently divided, as Butler (4) has shown, into two general types; first, discontinuous over comparatively limited areas by means of a succession of short jumps; second, continuous over large extents of land or water without interruption. Spread of these diseases in the Philippines comprises both types. It is chiefly local and discontinuous, involving short successive steps from field to field and region to region. To a limited extent it is also continuous, involving longer unbroken jumps from island to island over intervening seas. Spread of these diseases to

such distant maize-growing countries as the United States, however, must necessarily be continuous.

The Philippine Sclerosporas can be disseminated in three different stages in their development, as mycelium, as resting spores (oospores with adherent oospore wall), and as conidia. Each of these demands consideration in its relation to the various phases of local and of distant spread in the Philippines or to remote countries.

DISSEMINATION BY MEANS OF MYCELIUM

Mycelium, from its very nature can not be distributed by itself but must be carried with some part of the plant such as cuttings or seed. Infected cuttings do not, of course, enter into the problem as far as maize is concerned, but in the case of sugar cane and of the wild grass, *Saccharum spontaneum*, the Sclerosporas are spread to a limited extent by this means. At Bontoc in the Mountain Province the writer found in January, 1920, that almost all the numerous small patches of cane grown in and around the Igorote villages were heavily infected with an oogonial Sclerospora; and there was strong evidence that the indiscriminate use of infected cuttings had, at least in part, been responsible for its spread. This cane, however, probably because it is a thin, hard-stemmed, purple variety with a rather low yield, had not been distributed widely; and as far as the writer was able to determine the disease is restricted to the one locality. Introduction of this cane into the sugar-producing regions of Calamba, Pampanga, or Negros would be extremely dangerous. Except at Bontoc no instances of distribution of Sclerospora by mycelium in sugar cane were noted in the Philippines. The only other case of Sclerosporal infection of sugar cane seen by the writer was a single plant attacked by the conidial form, *Sclerospora spontanea* (*25*), and so weakened and stunted as to be useless for cuttings. It should be noted, however, that distribution of the Sclerosporas by this means is greatly to be feared, especially by remote countries. It was through the importation of cuttings infected by the mycelium of *Sclerospora sacchari* that this destructive disease was introduced into Formosa from Australia (*15*).[4]

In the case of the wild grass, *Saccharum spontaneum*, also, spread of Sclerospora is accomplished to some extent by infected cuttings. In certain provinces, especially Laguna and Pangasinan, it is a common practice to plant this grass by means of cuttings, as a binder along the newly made retaining walls between rice paddies. In those provinces the oogonial Sclerospora which has previously been mentioned (*25*) is very prevalent on the grass, and as the farmers, ignorant of the true nature of the disease, make no effort to select healthy stems, the mildew is frequently transplanted in the cuttings. As a result, in the newly grown grass borders of these fields the writer commonly encountered extensive infections which in location, age, and other characters gave clear evidence of arising from infected cuttings. Experimental plantings, using diseased stalks, developed similar extensive infections. In the case of plants of this grass parasitized by the conidial *Sclerospora spontanea* the writer also found experimentally that cuttings could carry the disease, but no instances of such transmission of the conidial stage were observed in the field.

[4] Since this was written the writer has learned from Mr. H. A. Lee, of the Philippine Bureau of Science, that *Sclerospora sacchari* has recently been introduced into the Philippines from Formosa in cuttings whose infection remained undetected through inspection in Formosa and Manila and persisted uninjured through precautionary dipping in Bordeaux mixture.

Mycelium-infested seed, in contrast to cuttings, apparently plays no part in the distribution of Philippine maize mildews. Although in maize the writer (*24*) has found Sclerospora mycelium in the seed coats and even invading the endosperm, a series of experimental plantings of such seed in all stages of development, and even with the diseased cob still attached, gave rise to no mildewed plants. Similar plantings of such seed from infected plants of teosinte (*Euchlaena luxurians* Schrad.), teosinte-maize hybrids, and *Saccharum spontaneum* gave like negative results. In the Javan maize Sclerospora, Palm (*16*), after careful histologic study and extensive plantings, found the same to be true. These facts are of interest, as it has long been assumed that seed-borne mycelium is a means by which Sclerospora is distributed. This assumption has been based on investigations carried out on *Sclerospora macrospora* Sacc. in which the oogonial phase alone is known, and on *S. graminicola*, in which the conidial stage is rare and the oogonial preponderates. It should be noted that in the cases recorded infection might well have arisen, not from seed-borne mycelium but from oospores which in both species are produced in vast numbers and were undoubtedly adhering to or present in the coats of the seed used. This explanation would apply to Peglion's observation (*17*) that grain from wheat heads infected with *S. macrospora* and showing mycelium in the outer coats produced, when germinated, abnormal seedlings with mycelium in their tissues. It would also apply to the case described by Massee (*14*) of an outbreak of Sclerospora in England on sugar-cane grown from seed from France, a case that is particularly interesting not only because it presents evidence for seed transmission, but also because the resulting downy mildew was *S. graminicola*, a species as yet unrecorded on cane.

DISSEMINATION BY MEANS OF OOSPORES

When the distribution of the Philippine Sclerosporas by oospores is considered it is impossible to state how much or how little is accomplished by these bodies, since unfortunately the exact role which they play is not at present understood. That they must be functional to some extent in enabling the disease to withstand unfavorable conditions or to spread over distances so great that conidia would be killed in transit seems logical and necessary. Yet, in so far as the writer was able to determine, oospores are never produced on maize in the Philippines. The conidial stage alone occurs on this host, persisting, although somewhat diminished, through unfavorable seasons, spreading disastrously in favorable periods, and causing widespread destruction throughout the islands, all apparently quite independent of any oogonial phase.

So far as their existence on maize is concerned one need consider the Philippine Sclerosporas only in the conidial stage to understand their persistence, their spread, and their destructiveness. But, on the other hand, on other gramineous hosts in the Philippines it is the oogonial stages that are predominately widespread, persistent, and destructive. On *Saccharum spontaneum*, for example, Sclerospora oogonia are extremely abundant, not only in the lowlands where the conidial phase, although rare on this host, is common on maize, but also in the mountainous interior of Luzon where conidia on either host do not occur or are so rare as to escape observation. Similarly, in this same mountainous region the wild grass *Miscanthus japonicus* and the cultivated sugar-cane show abundant infection with oogonia exclusively. Moreover,

this stage alone was found on wild grasses in remotely isolated uplands far from maize, and on islands two or three miles from shore.

The three oogonial Sclerosporas, although morphologically similar, occur on three different hosts and are occasionally found in widely separated localities. The conidial Sclerosporas are morphologically separable as two distinct species (*25*). It is impossible, at present, to understand what relationship, if any, exists between these conidial and oogonial stages. From the evidence at hand it is not apparent whether the conidial *Sclerospora philippinensis*, found on maize, sorghum, and teosinte, and inoculated to *Saccharum spontaneum* and *Miscanthus japonicus*, has as another phase in its life cycle the oogonial stages found on one or more of the three hosts mentioned above, and whether the conidial *Sclerospora spontanea* found on maize, *Saccharum spontaneum*, and sugar-cane, and inoculated to *Miscanthus japonicus* is related to these oogonial forms, or whether all are independent and unrelated.

The solution of this relationship necessitates germinating the oospores and securing conidial infection from inoculation with them. So far the writer's preliminary investigations of these points have resulted unsuccessfully, as have all the attempts reported in the literature by investigators of the genus from its founding by Schroeter (*22*) in 1879 to the present day. To be sure, in 1884, Prilleux (*18*) reported that M. Frechou, paramacien of Nérac, had seen the oospores of *Sclerospora graminicola* germinate by the protrusion of a hypha; but this investigation was not followed far enough to cast any light on the relationship of the two stages. Until this relationship is worked out, the part played by oospores in distributing and maintaining the downy mildews in the Philippines can not be understood.

When considering the Philippine Sclerosporas as a source of danger to the United States it is chiefly with the oogonial stage that we are concerned. Introduction probably would be accomplished by oospores, for as has been seen the fungi could not survive distant transportation except in this stage or as mycelium in sugar-cane or grass cuttings. If established in our country the Sclerosporas would survive the winter principally by means of the oospores which with their adherent oogonia are characteristically thick-walled and resistant. It is in this way that the closely related *Sclerospora graminicola* which thrives in the Tropics is able to live through the year in northern Iowa, Wisconsin, and Michigan. For these reasons the detailed study of the oogonial phases both of the Philippine Sclerosporas and of other species is one of the most pressing needs of future investigation.[5]

DISSEMINATION BY MEANS OF CONIDIA

It is the conidia which play the most important part in disseminating the downy mildews of maize throughout the Philippine Islands. From a knowledge of the details of conidium production and dispersal which

[5] It is of interest to note that since the foregoing was written *Sclerospora macrospora* has been found on wheat in the United States. Wheat plants attacked by this fungus were collected at Jordan, Ky., in April, 1921, by Mr. R. W. Leukel and identified by Dr. A. G. Johnson, who later found the same parasite on specimens of wheat collected in California in 1919. Subsequent investigation by the writer has shown that the Sclerospora is well established in the wheat fields of western Tennessee and Kentucky. This species has never been found in the Americas before, but in Italy, southern France, and other parts of Europe it has been known since 1895 as a disease sporadically destructive to wheat, maize, oats, rice, and various wild grasses. Its occurrence in Tennessee, far from wheat, on *Bromus commutatus* Schard., a grass introduced from Europe within comparatively recent years, possibly indicates that this host brought in the disease with it. In any case, this instance emphasizes the danger of the oogonial stage, the only one known in this species as a means of disseminating Sclerosporas.

have been given above, one is able to understand and adequately to explain the most important phases of the problem presented by these maize mildews in the Philippines. The gradual local spread of the disease in some regions and its sudden scattering over extensive areas in others, the apparent immunity of maize in certain localities and the destruction of the whole crop in others, the sudden appearance of the disease in areas hitherto uninfected and the equally rapid disappearance of long persistent infection elsewhere are all no longer baffling.

It should be noted, however, that the dispersal of conidia does not necessarily imply distribution of the downy mildews. Vast quantities of germinable conidia may be scattered by the breezes; but if they fail to reach susceptible parts of favorable hosts before being killed by drying, no distribution of the Sclerosporas will result. The following points must be borne in mind. To secure the production of large numbers of conidia there must be a heavy and lasting deposition of dew on the infected plants; to effect the dispersal of the conidia so produced there are required night breezes of favorable strength and duration; while to accomplish infection from the quantities of conidia thus scattered there must be available and accessible in the field, within carrying distance, young susceptible maize plants into whose dew-bathed buds and leaf axils the breeze-borne conidia may fall. These conditions are the limiting factors affecting distribution of the downy mildew through the agency of conidia. In the Philippines, climatic conditions and agricultural practices are such that these requirements are very generally fulfilled. As a result dissemination by means of conidia goes on, year in and year out, unhindered.

In achieving discontinuous, step-by-step distribution of the maize mildews, the conidia are preeminently important. At the beginning of each rainy season the downy mildews gradually spread in the newly planted maize from centers where the conidial stage has lived through the dry season on maize planted out of season or maintained under irrigation for forage. Along the edge of the broad Batangas plain, for example, small areas are kept in maize during the dry season at Sto. Tomas, where the proximity of Mount Maquiling insures unseasonable rains, and at Lipa where Mount Malepunyo serves similarly. At both places downy mildew persists on these plantings; and when the rainy season begins the new maize crop, exposed each night to large numbers of wind-borne condidia, becomes rapidly and extensively infected. Also, in the foothills of Mount Banahao, at San Pablo, Lilio, Nagcarlan, and Majayjay numerous densely and successively planted maize plots are maintained through the dry season to supply forage for the tough little ponies that are the chief means of transportation over the steep roads. In these plots the downy mildew is carried along to spread with disastrous effect to the more extensive plantings of the main maize-growing season.

Not only can the progress of the disease be followed in limited areas during early stages in the development of a single maize crop, but in the course of the growing season, which in some regions accommodates two or even three successive crops and occupies two-thirds of the year or more, spread over extensive areas can easily be traced. For example, in the Batangas plain and along the border of the Lake of Bay, where the progress of the maize mildew was followed closely during two years, it was observed to spread from the persistent foci of infection which have been mentioned and extend with ever-increasing severity and expansion

until finally in the last crop maturing in the early part of the on-coming dry season it had penetrated even to relatively remote fields and caused them heavy losses.

Whether the discontinuous spread of the mildews from island to island is due to the agency of conidia could not be settled by observation. Certain evidence, however, seems to indicate that such distribution does occur. The closely aggregated Philippine Islands are idealy situated for the dissemination of the downy mildews by wind-blown conidia. Of the entire 3,000 islands, about 1,000 are of sufficient size and importance to be worthy of agricultural consideration; and these are grouped in an irregular triangle extending about 1,000 miles from north to south. The 11 largest of these extend over more than four-fifths of this distance and comprise but little less than four-fifths of the total area. Yet the distance separating any one of these from the next is not over 20 miles—often much less. Moreover, their arrangement is such that typhoons and prevailing trade winds have a general lengthwise trend over them. Downy mildew of maize is already known from the great Island of Mindanao at the south, from the Visayan Islands in the center, and from Luzon at the far north. Seven of the 11 largest islands are known to harbor the disease; and in all probability investigation will show that it is established throughout the whole archipelago. That this broad distribution has been and is being accomplished, at least in part, by conidia blown from one island to another on night breezes seems very probable to the writer. Night winds, especially those which come in sudden gusts of considerable strength, often have a velocity of 10, 20, or more miles an hour; while the gales which distinguish nocturnal typhoons frequently reach hurricane velocity. Moreover, especially in the Visayan Islands, diseased maize is found in abundance on highlands which unobstructedly overlook the intervening seas. It seems probable that conidia swept by violent gales from such vantage points would take no longer to reach the maize fields of neighboring islands 10 miles distant than conidia borne on almost imperceptible air currents would take to drift from stunted plants to plates exposed 100 feet away.

Butler (*4*), in summing up the general question of wind distribution of fungi, concludes that—

> Several [cases] suggest that aerial spread is limited to a few miles, while other [fungi] are capable of bigger jumps, possibly 50 miles or (in the case of the oak mildew) even more.

The striking experimental evidence of Hesselman (*12*), on the other hand, shows that pollen grains much larger than conidia may be transported 30 and even 50 miles by breezes of moderate strength. It should be remembered, however, that pollen and the spores of most fungi are not so rigidly restricted in time or extent of distribution as are the downy-mildew conidia which can not survive drying and must be produced, scattered, and reach new hosts within relatively few hours of the night. The fact that the writer (*25*) has found two conidial Sclerosporas on maize apparently restricted to different groups of the Philippine Islands must also be considered. The writer inclines to the belief that dissemination of the maize mildews from island to island by means of conidia has taken or is taking place, but until further evidence has been obtained and the geographic distribution of these and other species has been worked out for the whole archipelago, no final decision on this point can be made.

It is clear, however, that the conidial stage of Sclerospora, at least in the Philippine forms, is far more complex in respect to conidium production and dispersal and far more important in disease dissemination than has been realized hitherto.

SUMMARY

In the Philippine Islands the two downy mildews, *Sclerospora philippinensis* and *S. spontanea* are in their conidial phases injurious parasites of maize. Investigation of the two species has cast light of certain hitherto obscure points in: (1) The production and dispersal of conidia, and (2) the relation of these processes to the local and distant dissemination of the diseases.

Production of conidia on infected plants is preceded by the establishing on leaves and sheaths of characteristically discolored areas that in outline and pallor are symptomatical of the disease. Production takes place chiefly and in greatest quantity on such areas which may occupy a relatively large proportion of the plant's surface.

Conidiophores never develop except from the stomata, at night, and when the surface is covered with dew or other moisture. From the mycelium in the underlying tissue, branches push through the stomatal pores, forming crowded groups of lobed and knob-like outgrowths. These elongate, developing successively into mature conidiophores, several of which are formed at each stoma during the course of the night. The conidia apparently are liberated by active ejection from the sterigmata, rather than by passive disjunction as has been assumed; but further investigation is necessary to establish this point. Nocturnal development of conidiophores in the usual amount of dew follows a relatively regular cycle, which, however, may be altered by rain or by drying winds.

Conidia are produced in vast numbers, even a small plant liberating a quantity sufficient to infect the neighborhood. The number produced on a plant during one night chiefly depends on the extent of the productive surface, on the number of stomata in this surface, and on environmental conditions. Approximate estimates of the number produced on representative plants of different ages are given.

Production of conidia on an infected plant is not limited to one night but may be repeated on successive nights when conditions are favorable over a period covering as much as 75 per cent of the total life of the plant. The duration of production in the case of several different varieties of infected maize is given.

Dispersal of conidia takes place necessarily at night. (1) It is accomplished chiefly by wind. Slight air currents are especially important; because, although local, they occur very frequently and at the time of maximum conidiophore production. Stronger breezes, usually near the sea and on broad plains, are effective because they occur often during maximum conidiophore production and cover considerable territory. Violent gales are not conducive to copious and general production of conidia but yet are important, because they rapidly sweep such few conidia as may be formed over great distances. (2) Dispersal of conidia is also accomplished by splashing. Drops of dew or rain falling on the conidiophore-bearing leaves, splash off, laden with conidia, and may reach young maize directly or may be carried to it by wind. (3) Dispersal is

also effected, though to a much less degree, by agents of minor importance, such as surface water, insects, and moist soil laden with conidia.

The genus Sclerospora may be disseminated in three stages of its development—as mycelium, as oospores, and as conidia. (1) Mycelium, in the case of maize itself, plays no part in disseminating the Philippine downy mildews. However, as mycelium in cuttings of other hosts, such as sugar cane and related grasses, these diseases are distributed in the Philippine Islands and thus could be brought to the United States. (2) The oospore stage is not found on maize in the Philippines. Whether the oogonial Sclerosporas occurring on sugar cane and two wild grasses in the islands are related to maize is not known. However, this stage must be responsible for at least some of the local and distant spread of these fungi and may possibly be involved also in the dissemination of the conidial phases on maize. (3) Not only during any one season but from year to year conidia are the most important means of accomplishing both locally and extensively the destructive spread of the downy mildews on maize throughout the Philippine Islands. Spread to the United States could not be accomplished by conidia unless these developed on plants imported alive. If the disease were introduced in any fashion, however, the conidia would be of the greatest importance in rapid dissemination.

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PLATE 1[1]

A.—Young maize plant (Mangonon White Dent) four days after the first symptomatic paling of the leaves. Conidiophore production which has already taken place on two favorable nights has begun characteristically on the distal portion of the upper leaves (*a*, *a*). The total conidiophore-bearing area at this early period comprises only a few square centimeters but as time goes on will continue to become increasingly extensive as the plant develops. $\times \frac{1}{5}$.

B.—A near view of the fifth to eighth leaves of a young maize plant (Chinese Waxy white), showing (at *x*, *x*) the etiolated stripes characteristic of the downy mildew. These markings had only begun to turn pale a little before dawn of the morning on which they were photographed, hence they were still inconspicuous and free from conidiophores. During the succeeding night, however, conidiophores were developed on the pallid areas. Natural size.

C.—Plant of maize (Guam White Flint) which, although only a little over 3 weeks old, is already producing vast quantities of conidiophores each favorable night from relatively extensive and conspicuously pale areas on its upper leaves and sheaths. $\times \frac{1}{4}$.

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[1] The photographs of Plates 1 to 6 were taken by the author.

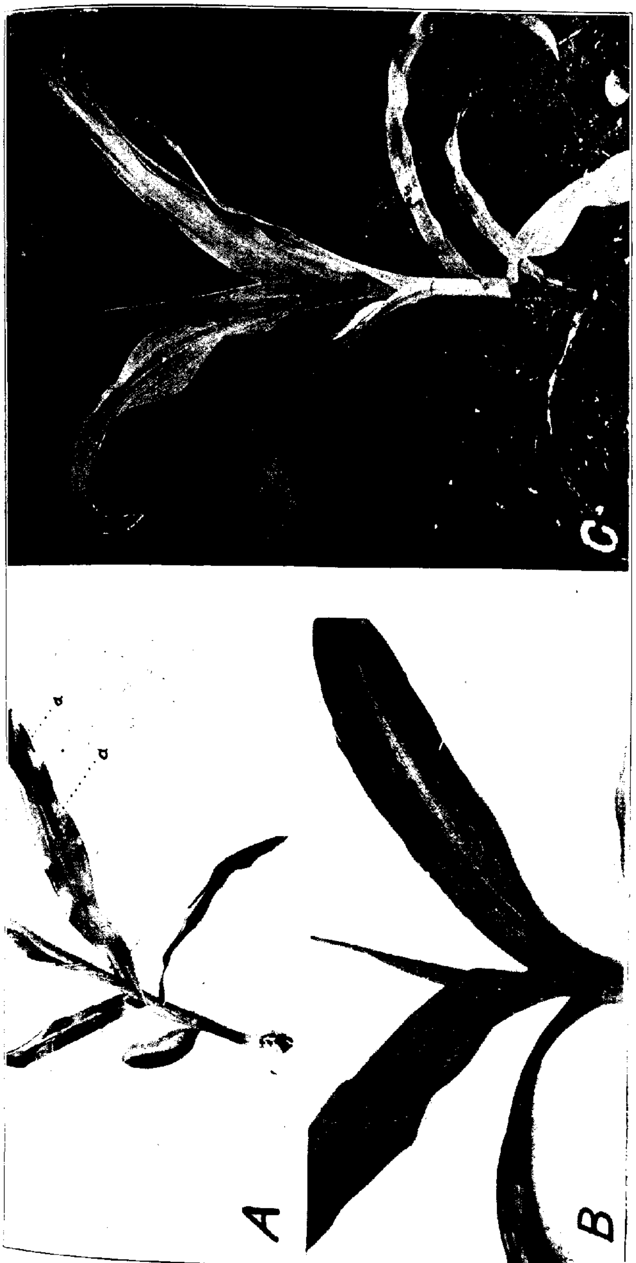
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PLATE 2

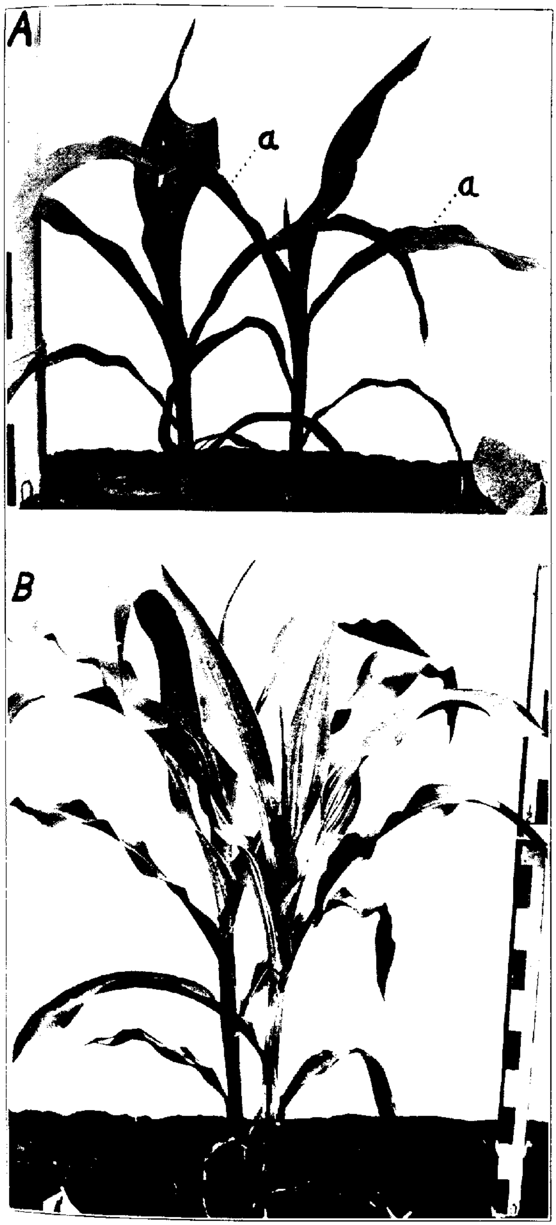
A.—Two young maize plants (Guam White Flint) 10 days after emergimg; the smaller and nearer one, healthy; the larger one already infected and showing (at *a*) the etiolated conidiophore-bearing areas characteristic of the downy mildew.

B.—The same plants about a month later. The conidiophore-bearing area which was inconspicuous and occupied only a few square centimeters on the leaves of the young plant is now very extensive and conspicuous and supports the production of vast numbers of conidia each favorable night. The meter stick, which is the same as that shown in A, is marked off in 5-cm. divisions.

PLATE 3

A.—Two plants of Manobo Waxy Maize 14 days after emerging; the one at the left healthy, the one at the right already infected with *Sclerospora philippinensis*, and showing etiolated stripes symptomatic of the disease. These stripes are relatively inconspicuous (*a*, *a*) and have a comparatively small area but are supporting abundant conidiophorz production from both their upper and their lower surface. The measure is marked in 5-cm. divisions.

B.—The same plants 16 days later. The etiolated markings which have been developed by the infected plant at the right are now relatively extensive and conspicuous and on them from both surfaces conidiophore production continues to take place in abundance. Measure as in A.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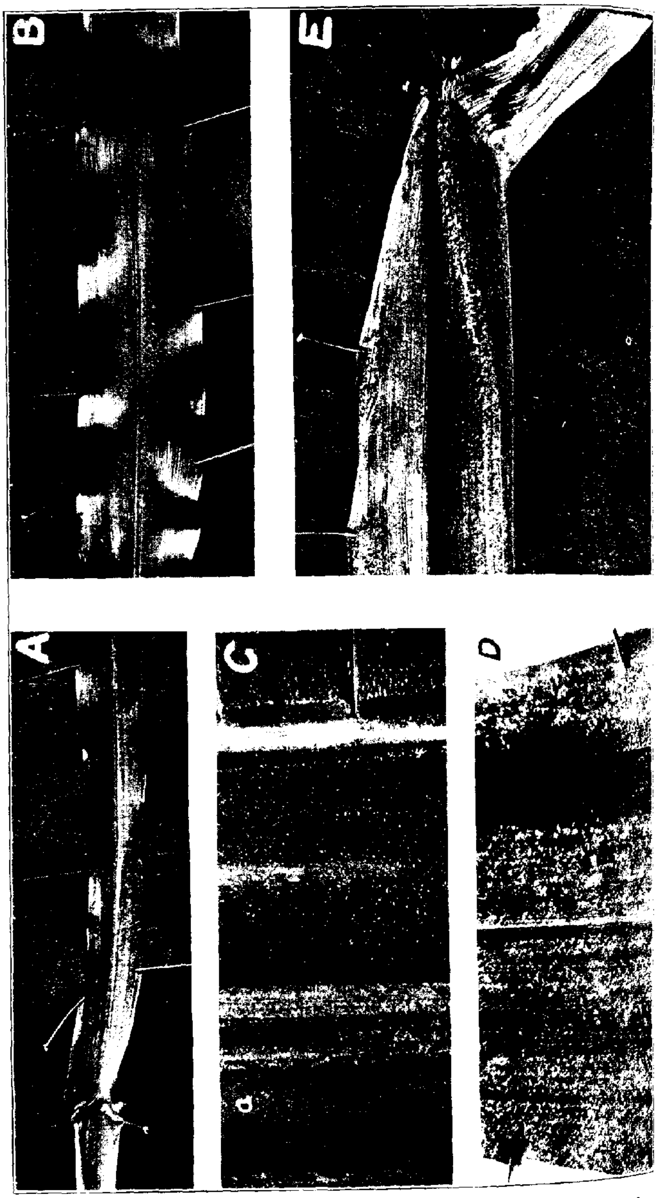
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PLATE 4

A.—Basal portion of the fifth leaf of a young plant (with seven leaves) of Pueblo White maize, showing recently established etiolated markings due to *Sclerospora philippinensis* which extend up from the very pale leaf sheath. Conidiophore production on this lower leaf had not as yet become abundant and conspicuous. Natural size.

B.—Distal portion of the sixth leaf of the same plant. The etiolated areas extend nearly to the tip of this leaf and on them conidiophores have already developed in abundance, especially along the midrib. Natural size.

C.—Portion of a leaf of Moro White maize from a plant heavily infected, with *S. philippinensis*. Conidiophore production is most abundant along the pallid stripes but also occurs scatteringly on the less conspicuously discolored areas between them and even on the midrib, *a*. $\times 2$.

D.—Portion of a leaf of Guam White Flint maize showing abundant production of the conidiophores of *S. philippinensis*. $\times 2$.

E.—Unrolled sheath of the uppermost leaf of a heavily infected plant of Moro White maize, showing conidiophores which were formed in the protection of this overlapping edge during a nocturnal storm that was so severe as to prevent spore formation on more exposed parts. $\times 1\frac{1}{2}$.

PLATE 5

A.—Deformed, virescent tassel of a plant of Cattete Brazilian Yellow Flint resulting from a severe attack of *Sclerospora philippinensis*. This plant showed the usual heavy production of conidia from its extensive leaf and sheath areas. In addition, from a large proportion of the surface of the abnormally developed bractlike outgrowths in this tassel conidiophore formation took place in abundance.

B.—Plant of Guam White Flint which, as a result of infection by *S. philippinensis*, developed an abnormal ear branch. This branch resulted from the elongation of the shank and the excessive growth of the husks of an ear which at first was apparently normal. Conidiophore production, after it had ceased on the main body of the plant, continued in abundance on the broad, leafy tips and the basal sheath portion of the husks of this branch, prolonging the period of spore production to a total of more than nine weeks. At the extreme left can be seen a healthy plant with a normal ear. The meter stick is marked off in 5-cm. divisions.


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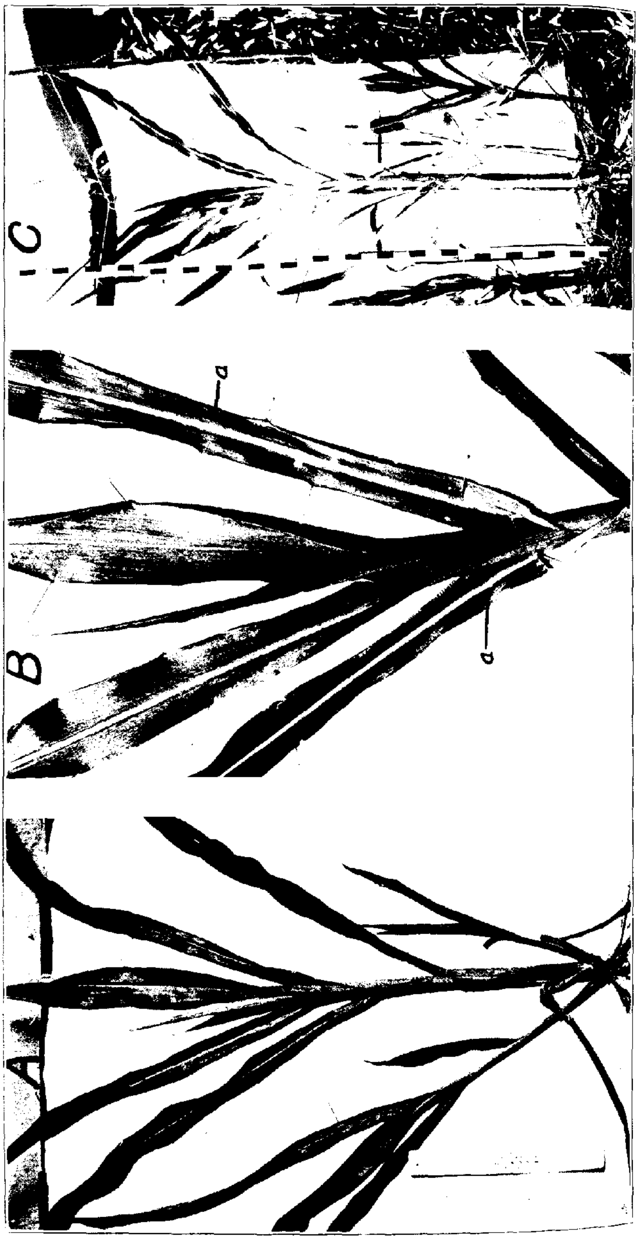
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PLATE 6

A.—A young teosinte plant infected with *Sclerospora philippinensis* and supporting abundant conidiophore production from relatively inconspicuous markings on the leaves, not only of the main shoot but also of the two lateral suckers by which the total spore-producing area in considerably increased.

B.—A closer view of the central shoot of A, showing the pallor of the conidiophore-bearing areas of the leaves and sheaths compared with the isolated stripes of normal green which still persist at *a*, *a*. As can be seen, the pallid portions occupy relatively a large proportion of the total leaf area, and since they support conidiophore production on both surfaces the total amount of production each night is large.

C.—An older teosinte plant growing as an escape in a field of Mexican June maize, from which it has become heavily infected with *S. philippinensis*. Although slender and spindling, the plant has a relatively large conidiophore-bearing surface and liberates great quantities of conidia each favorable night. Each division of the meter stick equals 5 cm.

PLATE 7[1]

A–D.—Stages in the development of the branches which start from the substomatal mycelium, push through the stomatal pore, and by forming lobed outgrowths give rise to the crowded group of knoblike buds that develop into the conidiophores. Maize (Guam White Flint) infected by *Sclerospora spontanea;* exterior view of the epidermis showing stages characteristically found from 10 to 11 p.m. A.—Stomata not yet invaded; its pores closed to a narrow slit. B.—From the substomatal mycelium, part of which can be seen dimly beneath the epidermis at *a* and *b*, many knoblike branch tips are crowding up through the stomatal pore. C.—A later stage in the growth of the knoblike branches, some of which have enlarged and pushed still farther out of the stoma. D.—A still later stage; the knoblike branches have grown into enlarged and lobed outgrowths which will elongate into the club-shaped conidiophore initials. ×700.

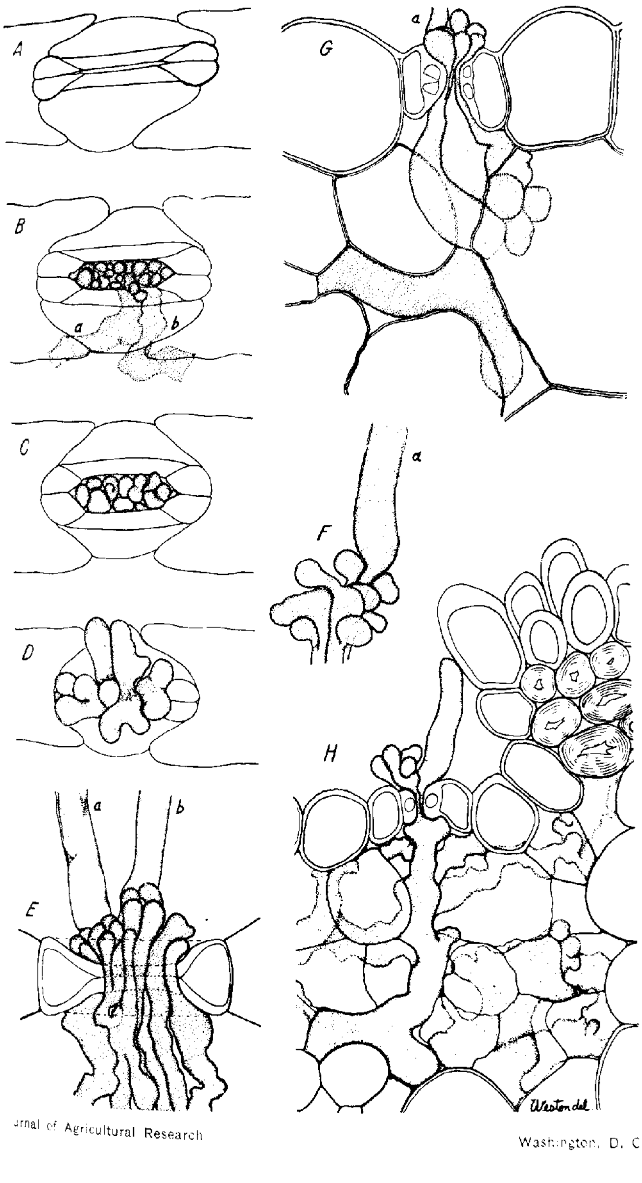
E.—A similar stoma seen in longitudinal section at a later stage in the development of the conidiophores. Above the group at *a* is shown the base of a conidiophore that has already lost its conidia and begun to shrink. At *b* is the base of another which is just maturing. Below are several knoblike outgrowths that will develop into conidiophores in their turn. From a longitudinal section of the same material cut at 2.30 a.m. ×700.

F.—A group consisting of outgrowths and their maturing conidiophores. Similar to that shown in E but dissected out from the surrounding tissue. The maturing conidiophore whose base is shown at *a* is figured in full in Plate 8, Q. 2.30 a.m. ×700.

G.—Portion of a cross section of the same material, showing in the substomatal chamber vigorous mycelial branches from which has arisen a group of knoblike outgrowths. At *a* is the basal extremity of a conidiophore already matured. 2.30 a.m. ×700.

H.—Portion of a cross section of *Saccharum sponatneum* leaf heavily infected with *Sclerospora spontanea*. From the stoma, which, in this grass, is deeply depressed and protected by ridges, has pushed out a small group of outgrowths one of which has begun to elongate into a conidiophore. Their relationship to the vigorous mycelial branches in the substomatal chamber can be traced clearly. 2 a.m. ×700.

[1] The drawings were made with the aid of a camera lucida and are from living material hand sectioned at night in water, the sections being immediately killed and fixed in osmic acid, stained, and mounted.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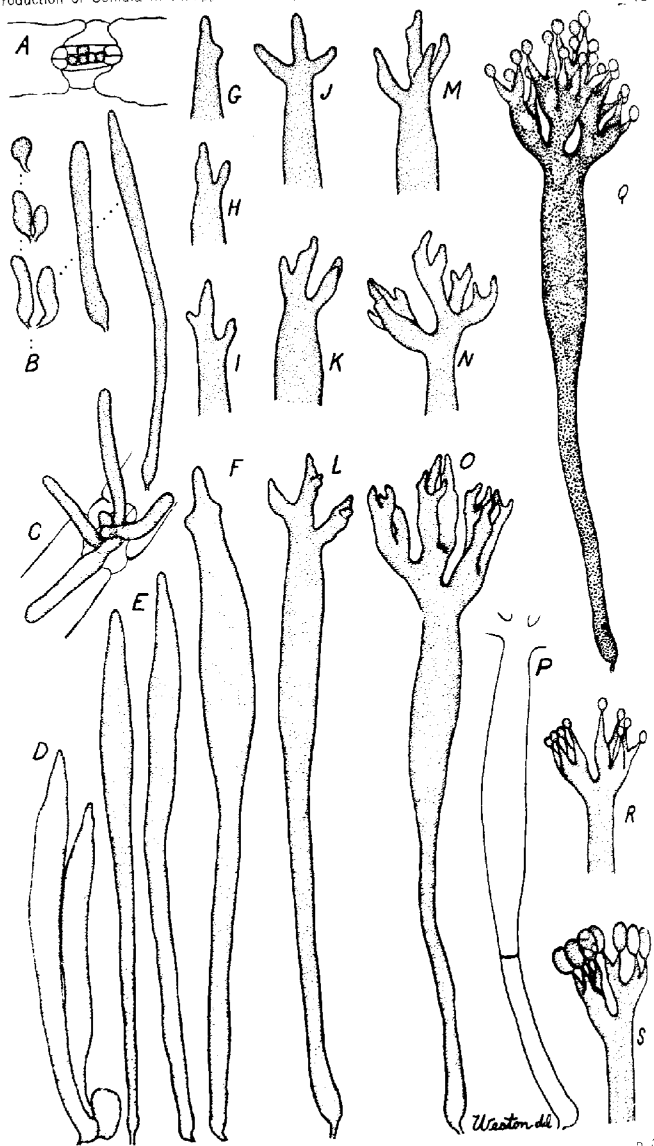
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PLATE 8[1]

A.—External view of stoma of maize, showing knoblike outgrowths from the internal mycelium throughout the distended pore. 10 p. m. ×375.

B.—Early stages in the development of conidiophores, showing gradual elongation of the small knoblike outgrowths into club-shaped initials. 10 to 11 p. m. ×375.

C.—External view of the stoma of maize (Barbados Yellow Flint) infected with *Sclerospore philippinensis*. Arising from the stomatal pore are four partly developed initials while a small knoblike outgrowth is pushing up between their bases. 11 p. m. ×375.

D.—Club-shaped conidiophore initials of *Sclerospora philippinensis*. Note the short, stocky habit and the indications that the basal cell will be short and stout. 11 to 12 p. m. ×375.

E.—Conidiophore initials of *Sclerospora spontanea* at approximately the same stage of their development. In comparison to those of the other species (D) they are longer and more slender in habit, especially in the lower portion that will be cut off as the basal cell. 11 to 12 p. m. ×375.

F.—Conidiophore initials at a later stage than those in E, showing the buds of the primary branches beginning to grow out. 11 to 12 p. m. ×375.

G-O.—Conidiophores showing later stages in the development of the branch system. 11 p. m. to 12.30 a. m. ×375.

P.—Conidiophore of *Sclerospora philippinensis* at approximately the same stage of development as the *Sclerospora spontanea* conidiophore (O). Note that the basal cell is shorter and is cut off by a cross wall at a relatively earlier age. 12.30 a. m. ×375.

Q.—Conidiophore showing conidia budding out from the sterigmata tips. Attempt has been made to show the contrast between the finely granular content of the sterigmata and the young conidia to the dense, coarse protoplasm of the main axes and branches. This conidiophore arose from the group of young knoblike outgrowths shown in Plate 7, F. 2.30 a. m. ×375.

R, S.—Later stages in the development of the conidia. 2 to 3 a. m. ×375.

[1] The drawings were made with the aid of a camera lucida from material which was scraped in dew from infected leaves at the time of maximum conidiophore production, immediately killed and fixed with osmic acid, stained, and mounted. The figures, with the exception of *C*, *D*, and *P*, show successive stages in the development of Sclerospora on maize (Guam White Dent) which had been inoculated with *Sclerospora spontanea* taken from *Saccharum spontaneum*. Figures *C*, *D*, and *P* are of *Sclerospora philippinensis* for comparison.

PLATE 9[1]

A–K.—Successive stages in the development of the conidia of *Sclerospora spontanea* from the tips of the sterigmata. Material found on maize (Guam White Flint). 2 to 3 a. m. ×1000. A–H.—The conidium enlarges gradually as more and more protoplasm flows into it from the sterigma. I.—At the junction of the sterigma tip and base of the conidium a hyaline zone is differentiated. J.—In this zone a wall is formed across the papilla of attachment of the conidium and likewise one across the tip of the sterigma. K.—Both walls are bulged outward increasingly by the turgidity of the contents.

L.—Conidium of *S. spontanea* which apparently has been broken off prematurely from the sterigma. Note the basal papilla. 2.30 a. m. ×1,000.

M.—Sterigma from which the conidium has been broken off prematurely. Most of the sterigmata on this conidiophore still retained their conidia in situ in the condition shown at J. Compare with P and Q. 2.30 a. m. ×1,000.

N.—Conidium of *S. spontanea* which, even after germinating, still indicates by the character of its papilla that it was broken off. 2.30 a. m. ×1,000.

O.—Conidium of *S. spontanea* that in the normal fashion has been thrown off from the sterigma. Note the bulged out papilla of attachment. 2.30 a. m. ×1,000.

P.—Sterigmata of *S. spontanea* from which the conidia recently have been thrown off. Note the characteristic shape of the bulged-out apices. The content has begun to show signs of disintegration. 2.30 a. m. ×1,000.

Q.—Sterigmata of *S. spontanea*. The characteristic shape of the tip is unchanged even in sterigmata of widely different sizes and shapes. 2.30 a. m. ×1,000.

R.—Sterigmata of *S. spontanea* long after conidium discharge. The content is disintegrating and the walls have begun to collapse, a condition characteristic of conidiophores like that shown in Plate 10, D. 4 a. m. ×1,000.

S.—Shriveled sterigmata with disintegrated content, such as are found on the dried-up conidiophore of *S. spontanea* shown in Plate 10, E. 3 p. m. ×1,000.

T.—Conidium of *S. philippinensis* that in normal fashion has been thrown off from the sterigmata. Note the bulged basal papilla. Material taken from maize (Barbados Yellow Flint). 2.30 a. m. ×1,000.

U, V.—Sterigmata of *S. philippinensis* from which the conidia have been thrown off. Note that the characteristic shape of the bulged tip is unaltered in sterigmata of different sizes. The same material as T. 2.30 a. m. ×1,000.

W.—Sterigmata of *S. philippinensis* from one of which the conidium has already been thrown off, while on the other the mature conidium is still retained in place. The same material as T. 2.30 a. m. ×1,000.

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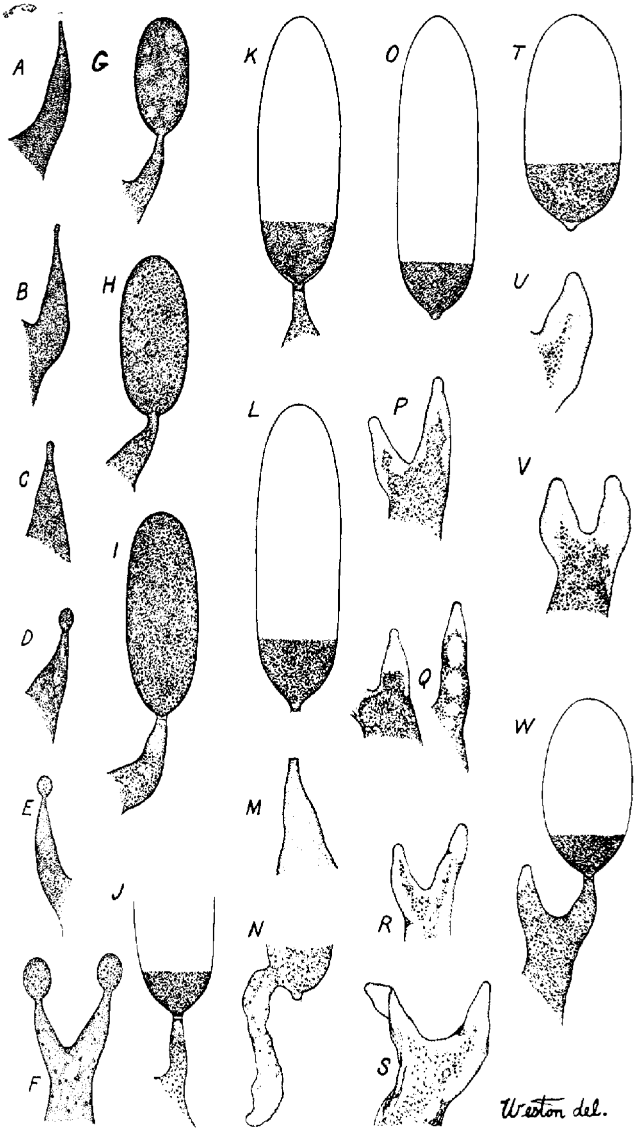
[1] The drawings were made with the aid of a camera lucida from material which was scraped in dew from infected leaves at the time of maximum conidiophore production, immediately killed and fixed with osmic acid, stained and mounted.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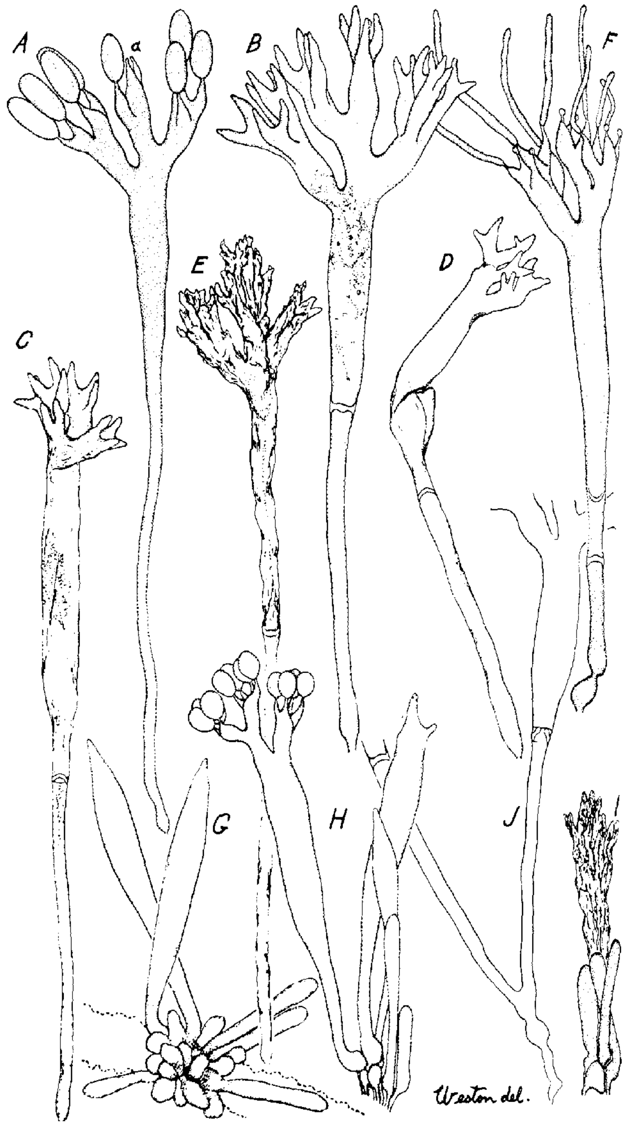
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PLATE 10[1]

A.—Conidiophore and maturing conidia of *Sclerospora spontanea* on maize (Guam White Flint). Note the truncate apices of the sterigmata at *a* from which the conidia have been broken off prematurely. 2 a. m. ×375.

B.—Similar conidiophore from which the conidia recently have been thrown off. Note that the apices of the sterigmata from which the conidia have been thrown are bulged out. Now that it has functioned the content of the conidiophore begins to disintegrate. 3 a. m. ×375.

C.—Similar conidiophore at a later stage; the sterigmata are little altered but the main axis is losing its turgidity and becoming shrunken. 4 a. m. ×375.

D.—Similar conidiophore still later. The sterigmata and branches have shrunk together and the main axis is shriveling. 5 a. m. ×375.

E.—Similar conidiophore after being dried on the leaf during the day to a wrinkled mummy that is hardly recognizable as the remains of the vigorous structure of the preceding night. 3 p. m. ×375.

F.—Conidiophore of *S. philippinensis* on maize (Barbados Yellow Flint) which shows an unusual formation of hyphae similar to germ tubes from sterigmata that had just begun to bud out conidia. Apparently the conidiophore, having inherent in its protoplasm potentialities for hyphal germination, has for some reason behaved as a multiple conidium. 2.30 a. m. ×375.

G.—Group of *S. philippinensis* conidiophores views obliquely from above, comprising a relatively large number of young stages and completely covering the stoma; from maize (Barbados Yellow Flint) at 2 a. m. ×375.

H.—Similar group of conidiophores, one maturing conidia, and the others in various earlier stages of development, teased out from the surrounding tissue of a leaf section of maize (Barbados Yellow Flint) at 2 a. m. ×375.

I.—Similar group of conidiophores, comprising three club-shaped conidiophore initials and one shriveled vestige of a conidiophore produced probably on some previous night, teased out from the stoma of maize (Barbados Yellow Flint) at 2.30 a. m. ×375.

J.—Unusual basal cell of forked structure serving as the base for two *S. spontanea* conidiophores from maize (Guam White Flint). 2.30 a. m. ×375.

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[1] The drawings were made with the aid of a camera lucida from material which was scraped in dew from infected leaves at the time of maximum conidiophore production, immediately killed and fixed with osmic acid, stained, and mounted.

MECHANICS OF INOCULATION WITH SUGAR-CANE MOSAIC BY INSECT VECTORS[1]

By E. W. BRANDES

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INTRODUCTION

It has been demonstrated with practical certainty that the only method for natural spread of sugar-cane or grass mosaic is through the agency of insects, acting as simple vectors or possibly as intermediate hosts.

It was established by the writer (*1*)[2] that *Aphis maidis* is able to transmit this disease. A considerable amount of confirmation of this result has now accumulated and notwithstanding some early skepticism it appears to be generally accepted. The mere fact of its ability to function as a vector, however, has been regarded by several investigators as of no great practical significance on account of the supposed scarcity of the insect on sugar cane. It was even suggested that *A. maidis* never occurred on sugar cane and that when colonized on cane plants the insects would die. On the contrary, other investigators have suggested that *A. maidis* is responsible for much of the natural transmission of mosaic in commercial plantings of sugar cane. In view of these divergent opinions, a review of experimental work on this subject is desirable.

REVIEW OF EVIDENCE ON INSECT TRANSMISSION

Taking up the published work in chronological order, Brandes (*1*) announced that *Aphis maidis* is able to transmit the disease to healthy plants after a previous feeding on diseased plants. It was stated in this paper that all species of insects used in the experiments were known to feed on sugar cane and that *A. maidis* in particular had been reported on sugar cane from practically all sugar-cane countries. Thus it was assumed that a certain amount of natural transmission in the field is traceable to this insect. The belief was expressed, however, that natural transmission is not restricted to *A. maidis*.

Smythe (*8, 9*) carried on a large number of experiments on insect transmission of mosaic at the Insular Experiment Station at Rio Piedras, Porto Rico. He reports successful transmission with a variety of insects. His results, however, are not convincing. The small number of experiments which he reports as successful are questionable, since in some cases his test plants were transplanted to an open field and left for a period greater than the minimum incubation period for mosaic before showing symptoms.

Tower (*10*), also working in Porto Rico, got only negative results from experiments in which he used sucking and chewing insects.

[1] Accepted for publication Nov. 17, 1922.
[2] Reference is made by number (italic) to "Literature cited," p. 283.

Ledeboer (*6*), in Java, announced that he had obtained results which indicated that *Aphis sacchari* as well as *A. maidis* could transmit the disease. In a later publication (*7*) he states that while his experiments with *A. maidis* had confirmed the work of Brandes (*1*), his preliminary experiments with *A. sacchari* were not now considered conclusive. He made the observation in this paper that *A. maidis* was noticed to be very abundant on *Panicum colonum* and *Paspalum sanguinale* in the sugar-cane fields and that the winged forms occasionally flew from these grasses to the sugar cane. He states further that these grasses are subject to the cane mosaic.

Bruner (*2*) published the results of careful experiments with a great variety of cane insects conducted at Santiago de las Vegas, Cuba. *Aphis maidis* alone was able to serve as a vector of mosaic. He states, however, that this insect does not attack sugar cane under field conditions and therefore can not be held responsible for dissemination of the disease in nature.

Wolcott (*11*), who studied insects in relation to this disease in Porto Rico, states in reference to *Aphis maidis:*

> The Corn aphis has not been found on cane in Porto Rico. It can not in any way be considered responsible for the transmission of mosaic disease of sugar cane under field conditions in Porto Rico.

In another publication (*12*) he says:

> . . . Mr. Smythe was unable to keep it [*Aphis maidis*] alive on sugar cane in Porto Rico. The experimental transmission by an insect which will live on sugar cane only under abnormal conditions [referring to the experiments of Brandes] proves nothing as to the insect responsible for transmission in the field, except to indicate the place on the cane plant where the insect must feed to cause the disease.

Kunkel (*5*), in Hawaii, performed careful experiments in which he confirmed the ability of *Aphis maidis* to transmit the disease, but was unable to demonstrate this ability on the part of *A. sacchari*. He also proves convincingly that the corn leafhopper (*Peregrinus maidis*) is able to transmit the disease from corn to corn but, strangely enough, not to sugar cane. Kunkel makes the important observation that a rapid spread of mosaic in sugar cane followed the weeding of a field. In the case cited, the field was infested with goose grass (*Eleusine indica*), some of which had the mosaic. *A. maidis* was abundant on the goose grass previous to weeding, and probably migrated to the cane at that time.

Chardon and Veve (*4*) conducted experiments in Porto Rico in which mosaic and healthy sugar-cane plants in a field were covered with cheese cloth, and *A. maidis* was introduced into the cage thus formed. The insects colonized on various grasses, including *Eleusine indica*, *Echinochloa colona*, and *Panicum barbinode*, which were growing as weeds in the cage. The weeds were then cut down and the aphids were observed to migrate to the cane. About 65 per cent of the healthy plants in this cage became mosaic during a period of 66 days, the first case appearing 14 days after the migration. In a second similar cage, into which no specimens of *A. maidis* were introduced, no cases appeared among the healthy plants. A somewhat similar experiment with a different species of plant louse (*Carolinaia* sp.) which occurs on the sedge, *Cyperus rotundus*, was reported in the same paper. Four out of six plants in the experiment became mosaic, but since no control plants appear to have been used it needs repetition under better controlled conditions.

A study of these results seems to afford abundant confirmation of the fundamental fact involved—namely, that *A. maidis* acts as a vector of sugar cane or, more properly, grass mosaic. Experiments with positive and convincing results have been performed in the United States, Java, Cuba, Hawaii, and Porto Rico. The fact also appears to be established that while *A. maidis* prefers other grasses to cane as a source of food, it frequently migrates to the cane in large numbers. Weeding of a cane field is evidently a prime factor in bringing about this result, but the present writer has observed infestation of sugar cane by *A. maidis* in Florida in fields which are never weeded. The conclusions of investigators who disclaim that any practical importance attaches to this insect as a vector of mosaic is based on negative evidence, and therefore can not be held to controvert these facts in any way. Evidence has been adduced indicating that two other insects, *Peregrinus maidis* and *Carolinaia* sp., may transmit the disease.

MECHANICS OF INOCULATION

In an endeavor to gain some insight into the method by which insect vectors inoculate plants with sugar-cane mosaic, a number of individuals of several species of sucking insects were killed *in situ*, embedded in paraffin, sectioned and stained. The insects used were *Aphis maidis*, the known vector of mosaic; *Peregrinus maidis*, the corn leafhopper reported by Kunkel (5) to transmit mosaic from corn to corn; and *Draeculacephala mollipes*, the large leafhopper which is so abundant on cane in this country, but which has failed to transmit the disease in repeated experiments. The latter insect was examined for comparative purposes, to determine if possible whether structural or functional feeding phenomena might inhibit its ability to transmit the disease.

It was found that various methods had to be devised for killing the various insects in the feeding posture and holding them so during all the steps of fixing, dehydrating, infiltrating, and embedding. With *Aphis maidis* the process was found to be comparatively simple. A young leaf of corn with a quiet feeding colony was clipped from the plant and thrust into nearly boiling Carnoy's fluid. The insects were apparently killed almost instantly, before they could withdraw their setae, and it was only necessary to transfer the pieces of leaf with attached insects through the succeeding reagents with utmost care in order to prevent agitation of the liquids and consequent dislodging of the insects. In the case of *Peregrinus maidis*, a colony of adults and nymphs on a corn leaf was cooled for 20 minutes at a temperature of 21° F., and when the insects were rendered inactive by cold the leaf was plunged into the hot Carnoy's fluid. A large percentage of these insects floated off but some remained attached. Specimens of *Draeculacephala mollipes*, comparatively large and heavy as well as active leafhoppers, were handled individually in small, moist chambers to the inside wall of which a piece of corn leaf had been pressed and held in a vertical position by means of gum arabic. The insect was gradually rendered inactive by cold while feeding, then suddenly pressed against the stationary leaf with the index finger and at the same instant covered with Carnoy's fluid as hot as could be borne by the finger. Since these insects possess very large and thick feeding setae, as compared with aphids, and therefore do not thrust the setae into the tissues to a great depth compared to the diameter of setae and weight of the insect, as in the case of the

aphids, it is readily seen that their position on the leaf after being killed is relatively insecure. They must be fastened in some way, and this was accomplished by using small drops of collodion as a cement to hold the feet in position. Since this material is soluble in absolute alcohol it was replaced with cellulose acetate when the insects were transferred to this reagent. The cellulose acetate, being soluble in chloroform, could not be used during fixing in Carnoy's fluid following killing. Sections 10μ and 12μ in thickness were obtained and were stained with safranin and gentian violet.

It was demonstrated that with *Aphis maidis* the beak is usually placed on the cuticle covering a stomate guard cell at the point where the cuticle is thinnest (Pl. 1, A) and the setae thrust into the latter by pressure. During this process a copious secretion is excreted at the end of the setae from the salivary glands. The secretion is colored brilliant red by the safranin. This secretion continues to pour from the tip of the setae as the latter passes into the deeper tissues, and forms the sheath described for other aphids by Büsgen (*3*) in his work on honey dew. Typically, the setae of *A. maidis* pass through the sub-stomatal cavity, then through the mesophyl cells either intercellularly or intracellulary, continuing between two cells of the starch sheath and finally into the phloem of the vascular bundle. (Pl. 1, B.) If the ligmified secondary thickening of the xylem elements are encountered, they get no farther in this direction. (Pl. 1, C, D.) In one section (Pl. 2, C) the preference of this insect for phloem is well illustrated, since an exploratory puncture leading to the xylem elements of the bundle is indicated by the empty sheath, while the setae which were partly withdrawn and again pushed forward are shown leading around the xylem and turning in the direction of the phloem. During the whole process the copious secretion from the insect pours into the practically uninjured and rapidly growing tissues of the leaf. The fact that it leads to the phloem cells, rich in substances of known nutritive value for microorganisms, seems especially significant. In all three sections of the series illustrated in Plate 2 the unusual amount of salivary secretion is clearly shown. It appears to the writer that this secretion is unquestionably the medium by which the infective principle of mosaic is carried into the plant. A more perfect mechanism for inoculation could scarcely be devised. There is no apparent wound reaction on the part of the plant; none could be seen in the phloem at any rate, and certainly none is apparent from the outside. Reaction to the virus itself does not cause great structural changes, as is well known. This phenomenon meets the requirements for successful inoculation in this disease in a peculiarly fitting way. It has been found that a definite, measurable quantity of "virus" is necessary. This would eliminate from consideration as an explanation the carrying into the plant of the scanty amount of virulent material adhering to the minute mouth parts. It is necessary to introduce the virus into rapidly growing tissues in the interior of the plant. Even in the much more easily communicated tobacco mosaic, a wound such as the crushing of trichomes seems necessary. Thus the anal secretion or honey dew falling on the normally unwounded surface leaves much to be desired as an explanation of the infection process. Aside, then, from the perfect adaptation of the setae to the requirements for successful inoculation, the phenomenon herein illustrated is the most likely explanation of the transfer of mosaic.

Sections of the leafhoppers, *Peregrinus maidis* and *Draeculacephala mollipes*, do not indicate that the phloem is especially sought during feeding. The evidence points more to the tracheae as the object of attack, but in the case of *D. mollipes* it is impossible to determine this point, since the relatively enormous setae are as great in diameter as the entire vascular bundles found in the young leaves where they prefer to feed. Nothing definite can be stated in regard to the feeding phenomena of *D. mollipes* which would exclude it as a possible carrier. All cells in the path of the setae, however, are crushed or engulfed in their entirety. There is some evidence of salivary excretion even in this large leafhopper, since a few traces of the brilliant red can be seen along the course of the puncture, but no definite sheath could be demonstrated. It would appear quite possible for this insect to penetrate to the vascular bundles by mechanical pressure alone instead of by the digesting or dissolving action of saliva which is quite necessary to account for the penetration of setae in the case of *Aphis maidis*.

These studies are being continued with virulent as well as nonvirulent aphids in parallel series in the hope that more light may be thrown on this problem and also on the etiology of the disease.

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PLATE 1

A.—Setae of *Aphis maidis* penetrating a young corn leaf. The black arrow points to the thin spot in the cuticular covering of a stomate guard cell where the setae entered.

B.—Continuation of the same setae shown in A. The white arrow points to the tip of setae lying in the phloem. This is the typical condition.

C.—Setae entering between epidermal cells.

D.—Same setae shown in C. The setae bundle has encountered a xylem element.


D
B
A


A
B
C

PLATE 2

A.—Setae of *Aphis maidis* penetrating the epidermis of young corn leaf.

B.—Same serae shown in A. This illustrates the copious salivary secretion.

C.—Same setae shown in A and B. An exploratory puncture to the xylem is indicated by the saliva sheath at the left. The setae bundle was withdrawn part way and again advanced toward the phloem.

RELATION OF POTATO SKINSPOT TO POWDERY SCAB[1]

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INTRODUCTION

The skinspot of the Irish potato tuber was first discovered, described, and named in England. It is said to be of considerable importance on seed potatoes, as the eyes may be injured and even killed by the causal organism, and this obviously would result in uneven stands and reduced yields. The same disease has been reported from Canada, and an apparently identical trouble has been described in Germany. It has also been noted on some shipments of Danish potatoes consigned to New York. Its presence in the United States was unknown until some wart-resistant varieties were imported from England and planted in the wart region of Pennsylvania. The progeny of these varieties showed a small percentage of skinspot. Further influx of infected material to this country is very likely to occur as long as the importation of foreign potatoes continues. It is therefore important to ascertain the nature of this disease and the possibilities of its spread in the United States.

SYMPTOMS AND ALLEGED CAUSES OF SKINSPOT

Owen (*7*)[2] stated very correctly that the external characters of skinspot are so distinctive that there is not much likelihood of confusing it with other spots. The appearance of the disease may be briefly described as follows: Round, raised, closed pustules frequently with a depressed border, single or in aggregates of varied configuration (Pl. 1, 2); dark brown or bluish brown outside and olive brown to brown inside. If the skin of the tuber is thick and rough, whether naturally or through the action of fungi or other causes, the spots are usually larger in diameter. Occasionally the pustules are lenticular or irregular in shape. Often the potato skin is attacked by *Spondylocladium atrovirens*, which gives it a glistening silvery appearance; but this is not characteristic of skinspot. In some cases the spots are not convex but are flat and only slightly raised and of considerably larger dimensions (Pl. 1, B). The disease is so typical and peculiar that no difficulty has been experienced by workers in recognizing it either on specimens or on photographs. The nature of the trouble, on the other hand, is less obvious, and the opinions of various investigators on this point are greatly at variance.

Carruthers (*1*) was the first to record what has been assumed to be, and presumably is, this disease. He found an abundance of fine mycelium in dead cells of the pustules but did not identify the fungus. However, his drawing of a diseased tuber covered with open pustules is very suggestive of powdery scab. No inoculation experiments are reported.

[1] Accepted for publication May 10, 1922.
[2] Reference is made by number (italic) to "Literature cited," p. 294.

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Pethybridge (*8*) observed the disease in Ireland and gave it the name of skinspot. Exposed cut surfaces of the pustules, when subjected to a moist atmosphere, produced a strong growth of a fungus identified as *Spicaria solani* Hart. He states that—

no cultures or infection experiments have been made in connection with this "skin-spot" and, therefore, it can only provisionally be regarded as caused by Spicaria.

Milburn and Bessey (*5*, p. 90–91) attributed an apparently identical disease which they called "blotches" or "pimples" to an organism referred to by them as *Spicaria nivea*. They obtained this fungus in every culture made by planting the inside of the pimples but failed to reproduce the disease by inoculation of healthy tubers with it.

A few years later Güssow (2), who previously worked with Carruthers, discarded the fungus theory of skinspot altogether and attributed it to unfavorable storage conditions which interfere with normal respiration of the resting tubers and result in the production of the symptoms of skinspot. He drew his conclusion solely from the observation that this trouble invariably occurred in badly ventilated storage cellars but reports no experimental work along this line.

About the same time O'Brien (*6*), describing Rhizoctonia injuries to potatoes in Scotland, included in his illustrations a photograph (*6*, Pl. V) which in all its characteristic features may be regarded as representative of skinspot. The author, however, uses it as an illustration of an injury caused by *Rhizoctonia crocorum* (Pers.) DC. and states in the text:

If such an affected tuber be moistened with water, numerous small blackish areas can generally be seen immediately below the surface which cause the skin of the potato to be elevated at such points. These areas are sclerotia.

Apparently without any connection with the English work on skinspot, Wollenweber (*10*) recently published in Germany a pamphlet on potato scab in which he describes a disease named by him "pustelfäule" and illustrates it with a photograph of the affected potato tuber which leaves no doubt as to the identity of this trouble with the English skinspot. This "pustelfäule," according to Wollenweber, is caused by *Phoma eupyrena* Sacc. He observed the mycelium of this fungus in the diseased tissues and obtained the organism in the majority of his isolation cultures. Further, upon inoculation of healthy tubers in needle pricks he produced "similar spots." He states very clearly that Spongospora was altogether absent in the diseased tissues.

The most extensive and quite recent study of skinspot was made by Owen (7). While using for the isolation work only those spots which had the skin unbroken, the author obtained in every culture a similar fungus which is described and named *Oospora pustulans*. In sections of the spots, the fine mycelium of this fungus, it is said, is plainly seen among the dead cells of the potato tissue. The hyphae are very numerous and for the most part hyaline early in April, when the fungus appears to be most vigorous, but later in the season they are very few and mostly brown. Skinspot, according to Owen, is a disease which develops in storage and is not usually visible until the early spring.

In concluding this bibliographical review it may be of interest to mention that the same disease has been locally known in British Columbia as pit Fusarium. The name itself explains its raison d'être—the disease is commonly found in pits and is believed by some to be caused by a species of Fusarium, presumably *Fusarium radicicola*. This erroneous opinion was based on a certain external similarity of skinspot pustules with some initial stages of the lenticel infection with the blackrot fungus as figured

by Pratt (*9*, Pl. 34, fig. 2–3), and also the occasional presence of *Fusarium* spp. in the pustules.

None of the workers mentioned in the preceding paragraphs gave conclusive proof for the support of their respective opinions as to the cause of skinspot. Their conclusions were based mainly on the presence of fungus hyphae within the dead cells and on subsequent cultures of the diseased tissues. The predominant organism in these cultures was assumed to be the cause of the trouble. According to the work of Owen (*7*) it was apparently *Oospora pustulans* in England, while according to the account of Wollenweber (*10*), it was evidently *Phoma eupyrena* in Germany. Güssow (*2*) and O'Brien (*6*) report no experimental work whatever. Carruthers (*1*) and Pethybridge (*8*) confined themselves to microscopic observations only. Milburn and Bessey (*5*) made inoculations of healthy tubers with *Spicaria nivea* but failed to reproduce the disease. Wollenweber (*10*) is content that he produced "similar spots" with *P. eupyrena*, but we know that an insertion of bits of mycelium of almost any facultative parasite in the needle pricks of tubers may result in the formation of brown pimples, externally resembling many a similar pathological condition. He gives no photographs and no description of the artificially produced Phoma spots by which others could judge as to the extent of their similarity with the skinspot pustules.

Owen (*7*) conducted a series of experiments both in the laboratory and in the field, which are described in sufficient detail. The results, however, were either entirely negative or not decisive. Only in one experiment a "small number" of tubers inoculated in the field developed the typical skinspot after they were stored in a cellar from lifting time until April. It should be noted that this experiment was carried on in unsterilized soil in the field, and that—

> much blight was present and a large number of the tubers had to be discarded.

It is not at all impossible that the latter fact may account for the absence of spots in one of the inoculated varieties and, perhaps, in the controls as well. On the other hand, the presence of "a few positive results" which became evident only in April after several months of storage in a cellar can hardly be regarded as proof that this small subsequent development was actually due to the artificial inoculation in July of the preceding year. The possibility of contamination either in the field during the growing period or later in storage is not at all excluded. It is not surprising, therefore, that the author arrives at the conslusion that—

> it will be necessary to wait another season before the results can be confirmed or amplified.

As yet, no further reports have been issued and the work, apparently, has been discontinued, at least temporarily.

TRUE NATURE OF SKINSPOT

While all the alleged causes of the skinspot disease lack convincing proof, a comparative examination of a large number of the affected tubers cannot fail to disclose a great similarity of these spots to the closed-pustule stage of powdery scab. The filamentous fungi invading the tissues of the pustules unquestionably complicate the conditions. Yet, whatever is the effect of this subsequent invasion, there is sufficient evidence that the original cause of this injury is the same as in powdery scab, ascribed to *Spongospora subterranea* (Wallr.) Johns. This evidence is presented hereunder.

MICROSCOPIC CONDITIONS

Kunkel (*3*) has observed the presence of the Spongospora plasmodia in the shrunken areas around the old sori. These the writer has not been able to detect in his sections of the skinspot pustules. The mature spore balls, naturally, are not to be found in the immature sori. Wollenweber (*10*) states that in his material "Spongospora was absent altogether." He does not explain what particular stage of Spongospora was sought in the "pustelfäule" spots or by what methods its absence was ascertained. The difficulty of demonstrating the plasmodium in the pustules of skinspot may find its explanation in the fact that at certain stages of its development it may, apparently, disappear from the spaces it has formerly occupied. This phenomenon has been noted by Kunkel (*3*) even in very young sori. He states:

Just beneath the epidermis can be seen the dark intercellular spaces which were previously occupied by the infecting plasmodium.

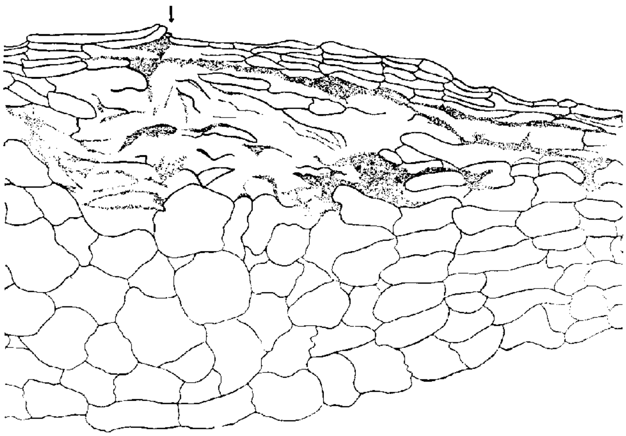

FIG. 1.—Semidiagrammatic drawing of a section through a typical skinspot pustule, showing the tapering spread of the infection between the cells. In several cases the infection has penetrated into the cells. A mass of broken-down cell walls is shown in the center of the spot. Some cells are slightly enlarged. The point marked by the arrow is probably the place of origin of infection. No hyphae have been observed in this spot.

(See also his plates XXXIX, XLI, and XLII.) Aside from this the general appearance of the vertical sections through the raised pimples of skinspot is very characteristic of the condition within the closed sorus of powdery scab (fig. 1). The center is usually filled with a disorganized mass of broken-down cell walls. This débris is surrounded on all sides by darkened intercellular spaces, indicating the path of infection. The infection spreads between the cells in the form of characteristic tapering projections. Occasionally a portion of the cell wall is destroyed and the infection extends into the cell. In some sections hypertrophied cells may also be observed. Fungus hyphae are entirely absent in a certain number of the pustules, which is fully in accord with the results of isolations (Table 1). Such spots may occasionally yield bacteria, but often when transferred to plates they remain sterile. In certain other spots the mycelium, although present, may be found only in very small quantity, dissociated from the apparent course of infection and obviously out of proportion to the area presumably killed by this mycelium.

The formation of new cork in the skinspot pustules on the border line of the diseased and the healthy tissues is also very significant and contradictory to the supposed progress of the disease. This process of cork formation is not invariably associated with the spots, though it may be seen in a considerable number of the specimens. Owen (7) gives a drawing of a section through a pustule of skinspot which shows distinct layers of new cork below the affected areas. Doubtless this phenomenon signifies that the parasite, after killing several layers of cells unhindered in its attack, finally ceased its activities, after which the host tissues erected a barrier between the dead and healthy regions. It is the course diametrically opposite to that which we would expect in *Oospora pustulans*, which is said to be invisible at lifting time but develops a considerable injury toward spring, or about the month of April. It should, therefore, at this time of the year be in its most active stage and not in a dormant state. On the other hand, the phenomenon of cork formation is quite in harmony with the progress of powdery scab. The active parasitic stage of the latter develops in the field during the growing period of the tuber. As observed by Kunkel (*3*), a certain degree of saprophytic activity of the fungus plasmodium develops in storage. This secondary infection results in dry-rot areas around the sori. However, it is, apparently, very slight with some spots and may be entirely checked with others. The cicatrisation in such cases is obvious.

ABSENCE OF THE SKINSPOT FUNGUS

During the years of 1920 and 1921 the writer had an opportunity to examine and study an abundance of material showing skinspot infection. This material was secured not only from England but also from other countries. It is believed, therefore, that a comparison of cultures isolated from this material will be of special value and interest. The various samples from which isolations were made are as follows:

1. Several tubers from British Columbia, crop of 1919, received in spring of 1920. Pustules only slightly raised above the surface of the tuber.

2. Five tubers from Dr. A. D. Cotton, England, crop of 1919, received in spring of 1920; sent upon request as representative samples of the skinspot disease.

3. Several lots of wart-resistant varieties shipped from England for work in Pennsylvania, crop of 1919, received in the winter of 1919–20. (Pl. 1, A to H, and Pl. 2, C and D, show some of these tubers.)

4. Tubers from various consignments of Danish potatoes received at the port of New York, crop of 1919, collected in early spring of 1920, (Pl. 2, B, shows one of these tubers.)

5. A sample of potatoes from New Castle, England, secured by Federal Horticultural Board inspectors in the spring of 1920.

6. A sample of potatoes from Edinburgh, Scotland, secured by Federal Horticultural Board inspectors in the spring of 1920.

7. A sample of potatoes from Ireland, secured by Federal Horticultural Board inspectors in the spring of 1920.

8. Two samples of the diseased tubers sent by Prof. J. W. Eastham, British Columbia, crop of 1920, received in the winter of 1920–21.

9. A number of tubers from the progeny of the infected English varieties (sample No. 3) planted in Pennsylvania in 1920, isolations made in the spring of 1921.

10. Tubers showing closed-pustule stage of powdery scab collected by the writer at Vancouver, B. C., in the fall of 1920. They were thoroughly washed after digging and stored for winter in a laboratory refrigerator. Isolations were made in March, 1921. (One of these tubers is shown in Pl. 3, A.)

11. Irish Cobbler tubers from Maine, crop of 1920, showing immature sori of powdery scab. Isolations were made in the winter of 1920-21. (One of these tubers is shown in Pl. 3, B.)

12. The same material as in the preceding sample, but isolations were made from mature sori.

Isolations were made from each of these samples and the results are presented in Table I.

TABLE I.—*Results of isolations from the skinspot pustules and the sori of powdery scab*

Number of the sample.	Number of spots from which isolations were made.	Number of spots which yielded no growth.	Number of spots which yielded *Oospora pustulans*.	Number of spots which yielded miscellaneous fungi and bacteria.	Remarks.
1	27	3	1	23	6 spots yielded *Fusarium* spp.
2	60	3	49	8	3 out of 5 tubers (42 spots) yielded exclusively *Oospora pustulans*.
3	15	5	6	4	No isolations were made from the open pustules shown on the photograph.
4	24	2	10	12	Samples showed no mature sori of powdery scab.
5	6	0	5	1	Sample similar to the preceding one in appearance.
6	6	0	3	3	Do.
7	6	0	6	0	Do.
8	34	2	6	26	1 sample (12 spots) yielded no culture of *Oospora pustulans*.
9	60	0	0	60	The predominant culture in this case was *Colletotrichum atramentarium* (Berk. and Br.) Taub.
10	12	10	0	2	1 *Phoma* sp. and 1 *Verticillium* sp.
11	12	7	0	5	Only 1 sorus yielded *Phoma* sp.
12	31	3	0	28	16 sori yielded *Phoma* sp. and the remainder miscellaneous fungi.
Total of 1 to 9 (undoubted skinspot).	238	15	86	137	*Oospora pustulans* 36.1 per cent.
Total of 2, 3, 5, 6, and 7 (English material).	93	8	69	16	*Oospora pustulans* 74.2 per cent.
Total of 1, 4, 8, and 9 (American and Danish material).	145	7	17	121	*Oospora pustulans* 11.72 per cent.

These results of isolations made from various lots of material in 1920 and 1921 show that *Oospora pustulans* is by no means the only invader of the skinspot pustules, since it comprised 36.1 per cent, or slightly above one-third, of the total isolations from the undoubted skinspot material.

Nor is *Phoma* sp. alone in attacking the *Spongospora* sori in Maine. In addition to a certain proportion of sterile plantings, that is, those which yielded neither fungi nor bacteria, a considerable percentage of organisms other than *O. pustulans* was obtained from authentic skinspot material. Especially striking is the complete absence of the supposed skinspot fungus from the typical pustules of the disease developed on potatoes grown in Pennsylvania. The largest number of *O. pustulans* cultures was isolated from the tubers grown in the British Isles (69 out of the total number of 86 *O. pustulans* cultures, or 74.2 per cent, of all the plantings made from English material). A somewhat smaller proportion was obtained from Danish specimens and a still smaller one from the samples received from British Columbia. None was isolated from the skinspot pustules which developed on potatoes grown in Pennsylvania.

It is not surprising that plantings of the diseased tissues did not yield *Spongospora subterranea*. So far only one worker (*3*) has reported success in culturing this organism; and, furthermore, these cultures were obtained by germinating the mature spore balls which are not yet present in the closed sori.

That a considerable number of the true powdery scab sori are likely to become invaded by some secondary parasite is not a new discovery. Previous studies of this disease in Maine revealed the fact that even in good storage conditions 30 to 75 per cent of the tubers infected with powdery scab suffer from a secondary rot due to *Phoma tuberosa*. The colored plate accompanying the paper cited here (*4*) shows two tubers with a number of closed sori. The latter have a depressed border and a raised center corresponding to the characteristics noted in connection with skinspot in England; but the organism responsible, at least in part, for this condition in the latter country is apparently *Oospora pustulans*. In Germany, Wollenweber's (*10*) work indicates, that a similar effect is produced by a species of Phoma different from that occurring in Maine. The principal invader of the skinspot pustules in Pennsylvania appears to be *Colletotrichum atramentarium*. This predominance in this connection of a certain species of fungus in a given country is an interesting phenomenon worthy of further study. It raises a question as to the rôle of climatic conditions in developing an intricate relationship within the fungus world, and in this case relationship between the powdery scab parasite and the secondary invader. It does not, however, solve the problem of the original cause of skinspot. It has long since become a commonplace in the science of plant pathology that the association of a certain fungus with the dead host tissue does not necessarily signify that it is the primary cause of death. It may as well denote any one of three other possibilities, namely, a secondary invasion of a parasitic nature, a saprophytic existence on the dead stratum, or a parasitic attack on the primary parasite. *Fusarium* spp. isolated by the writer from the skinspot pustules are, undoubtedly, secondary parasites, while such miscellaneous fungi as *Penicillium* sp. or *Actinomyces* sp. probably live there saprophytically. The part which is actually played in the potato tissues by *O. pustulans* as well as that by *Phoma eupyrena* or *C. atramentarium* remains as yet to be shown. The writer's laboratory inoculations of healthy potato tubers with *O. pustulans*, like those reported by Owen (*7*), gave entirely negative results.

It is not difficult to culture *Oospora pustulans* whenever the spots are actually invaded by this fungus. Therefore, the disagreement of the various investigators as to the cause of skinspot does not by any means

signify their inability to secure this organism in culture, were it always present in the diseased tissues. It is merely a reflection of the actual state of things. Their conclusions were determined mainly by the prevalence of one or the other semisaprophytic fungus in their cultures or by the absence of any apparent growth, as the case might have been. So far as the primary cause of the trouble is concerned, they were dealing with an organism which is far from being readily culturable by our usual laboratory methods.

VARIOUS STAGES ON THE SAME TUBER

While it is possible to single out many tubers which are covered only with the closed pimples to which the name skinspot is applied, yet in the examination of large quantities of infected material one is likely to find a certain number of specimens, varying perhaps with the seasonal conditions, which show all gradations in the development of the sori. It is rather difficult and sometimes impossible to determine whether the closed pustules in such cases should be classed as skinspot or powdery scab (Pl. 1, E to H). Like difficulty may be encountered in an examination of some unquestioned powdery scab material, grown, for instance, in Maine or Canada (Pl. 3, A, B, C).

An interesting question arises as to why in certain years there is an abundance of immature sori of the Spongospora scab. Theoretically speaking, this condition may be due either to an early check in the development of the disease or to a late infection. Both may be brought about by drouth and aided by the varietal response of the tubers. It is probable that late varieties with a later formation of tubers are more likely to show undeveloped powdery scab pustules, especially under adverse conditions.

During the latter part of September, 1920, the writer visited Sea Island and Lulu Island in British Columbia. A number of fields were examined on which it was said skinspot infested crops were produced in the preceding years, but no signs of the disease were noticed. Neither was the well-developed stage of powdery scab common. The local workers explained that the last two seasons had been very dry and in consequence powdery scab was practically eliminated. On the other hand, 1919 was the year when skinspot made its pronounced appearance and was noticed in abundance by United States inspectors and pathologists.

An early stage of Spongospora infection is usually not conspicuous at harvesting. The infected spots are not dark in color until they are exposed to the drying which takes place after lifting. During the long period of storage the tubers gradually lose moisture and shrink. The hard, dry, dark pustules then become elevated, especially when they become invaded by saprophytic or semisaprophytic organisms.

DISCOLORATION AND ARRANGEMENT OF THE SKINSPOT PUSTULES

Upon lifting the epidermis the majority of the spots show a peculiar chocolate-brown or olive-brown discoloration of the affected areas. This discoloration has frequently been described in the literature as very characteristic of powdery scab. It is not known to be so constantly associated with any other disease of the potato tuber. The arrangement of the pustules over the skin is likewise very suggestive of the Spongospora scab. Although often scattered without any definite order (Pl. 2, D), as is also the case with indisputable specimens of powdery scab

(Pl. 4, A), they are frequently arranged in a certain peculiarly regular manner, as for instance, in the form of bands or stripes (Pl. 1, A; Pl. 2, B, C), rings (Pl. 1, C, D) and aggregations, particularly at the stem end (Pl. 2, A; Pl. 3, A). All this is very typical of powdery scab (Pl. 4, B, C, D).

GEOGRAPHICAL DISTRIBUTION OF SKINSPOT

It is a striking "coincidence" that this disease occurs only in those countries, or in those sections of the countries, where climatic conditions favor the development of powdery scab. It has not yet been reported from those parts of the world where the latter is unknown. Thus, it is prevalent in the British Isles, exists in Denmark and Germany, and is established in parts of Canada. Occasional specimens showing closed pustules were observed in Maine, and they were always identified as the immature stage of powdery scab. It has developed recently to a slight egree in the elevated regions of Pennsylvania. No other similar trouble as been discovered in any other section of the States in spite of a coninuous influx of skinspot infected potatoes from Europe. This is exactly he behavior of powdery scab. The writer planted a large quantity of *ospora pustulans* material at Arlington Farm, Va., in 1920, but no spots ere observed on new tubers either at harvest time or in the spring of 921. The same negative results were obtained with tubers planted in he greenhouse at Washington, D. C., and grown in various kinds of soil nd under various conditions of moisture. Although the powdery scab ori may develop in Washington greenhouses, this is known to take place nly when tubers with the mature spore balls are planted. It has not et been ascertained whether the organism in the closed pustule stage an readily, if at all, become a source of infection of the new crop.

SUMMARY AND CONCLUSIONS

Evidence is submitted in the preceding paragraphs which show that:

(1) Neither *Oospora pustulans* nor any other filamentous fungus or environmental conditions mentioned by previous authors in connection with skinspot are proved to be the primary cause of the disease.

(2) *Oospora pustulans* is prevalent in England, and probably in some geographically proximal countries, but is either rare or entirely absent in the skinspot material grown in America.

(3) The various peculiar characteristics of the skinspot disease, as well as its geographical distribution are fully in accord with our present knowledge of powdery scab.

(4) Certain of these peculiar features can not be satisfactorily explained apart from the identity of the two diseases.

(5) The closed or immature sori of powdery scab present no apparent difference from the pustules of skinspot.

(6) The fungi associated with the spots in question are mainly secondary invaders developing during the storage period.

(7) These secondary invaders do not belong to one species, nor even to one genus, but vary with the country, their prevalence being determined perhaps by climatic conditions and the flora of the soil.

(8) Their presence in the sori of powdery scab is altogether unnecessary to give them the appearance of skinspot.

This evidence leads us to the conclusion that the skinspot pustules are essentially and primarily the closed or immature sori of powdery scab.

They are, however, frequently invaded during the period of storage by various filamentous fungi, in part saprophytes. In the light of this conception of skinspot we can understand the failure of this disease to spread in the United States in spite of numerous and continuous importations of infested material. We may further rest assured, so far as our knowledge of powdery scab goes, that the skinspot stage of the latter can never become a serious menace to the potato industry in this country. The term skinspot has no right to existence, except perhaps for practical convenience as a designation of a certain stage of powdery scab.

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PLATE 1

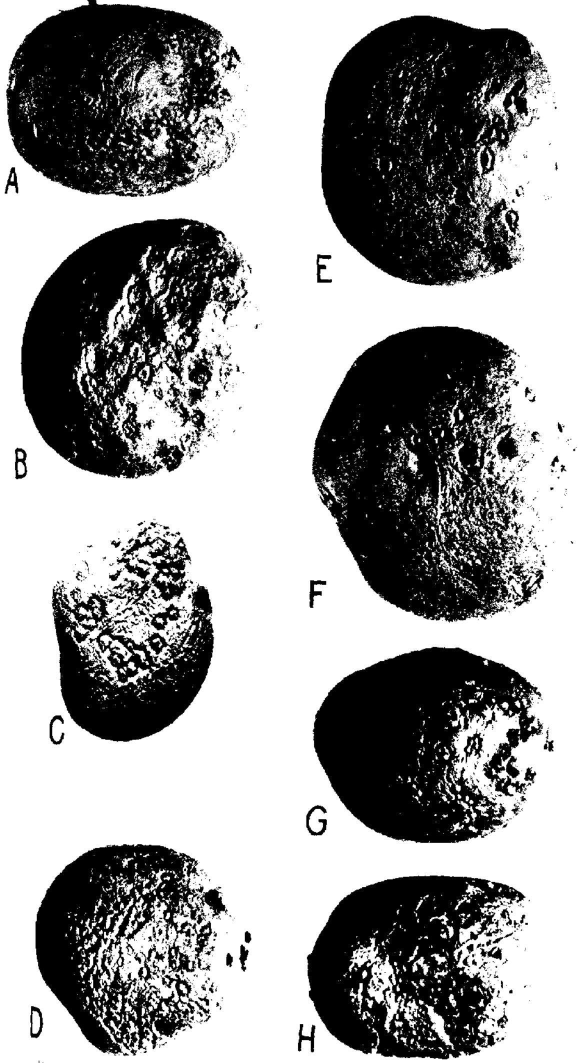
Various stages and arrangements of the typical skinspot pustules. Some tubers show mature sori of powdery scab also. All eight specimens were grown in England.

A.—Stripe arrangement of the pustules.

B.—Perfectly flat spots.

C, D.—Circles of the sori.

E to H.—Mixed stages of maturity of sori.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